RD-1453
TXR-5421

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

005421

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Dinoseb (2-sec-butyl-4,6-dinitrophenol) Registration Standard

TO: Richard Mountfort/James M. Stone
Product Manager (23)
Fungicide-Herbicide Branch
Registration Division (TS-767)

FROM: James N. Rowe. Ph.D.
Section V, Toxicology Branch
Hazard Evalution Division/HED (TS-769C)

James N. Rowe 6/6/86

THRU: Laurence D. Chitlik, D.A.B.T.
Section Head, Section V
Toxicology Branch/HED (TS-769C)

W. Testera for L. Chitlik 6-6-86

and

Theodore Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Attached is the Toxicology Chapter for the Dinoseb Registration Standard. Included are the following:

1. Summary of toxicology data for dinoseb.
2. Data gaps.
3. Tolerances and Tolerance reassessment.
4. Discussion of toxicological issues of concern.
5. Harrison tables.
6. Biblio_raphy.
7. One-liners update.
8. Study reviews.
9. Published tolerances with new ADI calculation using a provisional limiting dose.

Due to the potential oncogenicity of Dinoseb, the Toxicology Branch Peer Review committee met to discuss and evaluate the toxicology data submitted. Summary findings of the peer review meeting are included in as Appendix VI to the Registration Standard. As the new teratology studies are received(which you indicated have been submitted to the Agency), they will undergo expedited review and will be forwarded to you as an addendum to the Registration Standard with any appropriate changes in the data requirements for the Harrison tables.

005421

INDEX FOR TOXICOLOGY CHAPTER

005421

ACUTE TOXICITY

81-1 Acute oral

There are a number of acute oral toxicity studies for dinoseb.

In a study in rats, guinea pigs and chicks, dinoseb resulted in $LD_{50}$s of 40, 25 and 26 mg/kg, respectively, which places the compound in Tox Category I (MRID 90370). In mice, a $LD_{50}$ of 41 mg/kg was determined (Tox Category I) (MRID 60749). Intubation of a triethanolamine salt of dinoseb produced in rats, guinea pigs and chicks $LD_{50}$ values of 114, 88, and 70 mg/kg, respectively (Tox Category II) (MRID 67698). Finally, technical dinoseb (99% purity) gave a "survival" dose of 0.005 g/kg and a "lethal" dose of 0.05 g/kg in rats (MRID 90374).

These studies place technical Dinoseb in Toxicity Category I for acute oral toxicity. Although all of the acute oral studies are Core Supplementary data, for reasons specified in the study reviews, they appear to be adequate for determining the acute oral toxicity category for labeling purposes.

81-2 Acute dermal

An acute study with ethanolic solutions of dinoseb(99.1 to 99.7% purity), applied to guinea pig skin, was reported to give a "survival" dose of 0.1 g/kg and a "lethal" dose of 0.5 g/kg when applied to guinea pig skin (MRID 90374).

The acute dermal study was Core Supplementary for reasons specified in the study review.

81-3 Acute inhalation

No acute inhalation studies performed with technical dinoseb were found.

81-4 Primary eye irritation

No acute primary eye irritation studies performed with technical dinoseb were found.

81-5 Primary dermal irritation

No primary dermal irritation studies performed with technical dinoseb were found.

81-6 Dermal sensitization

No dermal sensitization studies performed with technical dinoseb were found.

81-7 Acute delayed neurotoxicity

There is no structural analog evidence to suggest that Dinoseb is a neurotoxic compound.

Acute toxicity data gaps include: acute dermal, acute inhalation, primary eye irritation, primary dermal irritation and dermal sensitization since no studies were found for these. An acute delayed neurotoxicity study is not required.

005421

SUBCHRONIC TOXICITY

82-1 Subchronic oral:

Dinoseb was fed to rats (25-30 days old) in the diet for six months at doses (weight %) of 0.00, 0.005, 0.01, 0.02, and 0.05% (Spencer et al., 1948; MRID 90374) in groups of ten/dose level. No cataracts were formed at any doses tested. The highest dose (0.05%) resulted in rapid weight loss with four of the ten rats dying by day thirteen of treatment; the remaining six animals were sacrificed at day 21 and revealed marked emaciation with an average blood urea-N concentration of 55.0 mg per cent. Microscopic examination revealed slight degenerative changes in the renal tubules and slight cloudy swelling of the liver. Examination of the growth curves for dinoseb-treated rats indicated that there was a small but consistent depression in body weight gains over the period of the study at the 0.02% dose level (3-8% below controls). There was a small but statistically significant increase in liver weights but other organ weights(kidney, heart or testes) were not affected by dinoseb administration. No discernible toxic effects were reported during the study, including BUN, weight loss, or at necropsy, at the 0.005 and 0.01% dose levels. This study had numerous deficiencies and was designated Core Supplementary.

Data on repeated feeding of Pekin ducklings was submitted with the study presented above(Spencer et al.,1948; MRID 90374). Ducklings were fed 0.25% for 3 days, 0.10% for 4 days or 0.03% for 8 days (weight % in diet). Pekin ducklings were very susceptable to the acutely toxic effects of dinoseb at doses of 0.25, 0.10 and 0.03% and the mid and low doses resulted in some of the birds developing cataracts (1/0.10%, 2/0.03%). In comparison, 2,4-dinitrophenol (0.25%) produced 100% bilateral cataracts after only a 24 hour period on the experimental regimen. This exposure period is too short for this to be considered a subchronic study. It had numerous deficiencies and was designated Core Supplementary.

A 91-day feeding study with Dinoseb in beagle hounds was performed at several dose levels(McCollister et al.,1967: MRID 114962): 0, 0.005 and 0.01 % Dinoseb; due to an acceptance problem, a fourth group was fed 0.005% for five days, raised to 0.01 % for four days, 0.015% for three days and maintained at 0.02% for 79 days; a fifth group was fed 0.02% for 26 days, and increased to 0.03% for 65 days. In light of the observation that all animals had been previously exposed to dinoseb, apparently through a treatment of the animals for hookworm, and that significant amounts of dinoseb were found in serum prior to commencement of exposure to the test compound, valid conclusions from the study regarding dinoseb's toxicity cannot be reached. It should be noted that vitreous opacities were seen toward the end of the study (82 days) in three of the animals, 1 male dog at 0.01% and 2 male dogs at 0.03%, suggesting that the eye may be a potential target organ for dinoseb--an effect previously noted in the Pekin duck. This study is classified as Core Invalid due to the major deficiency noted above and others.

A dietary feeding study was performed at several dosage regimens and durations in White Pekin ducklings (Tucker et al., 1967: MRID 114963): Experiment I: dietary concentration of 0, 1, 3, 10, 30, 60, 100, 200, 300, and 1000 ppm; Experiment II: 0, 100, 200, 300, and 1000 ppm; Experiment III: 20, 40, 60, and 80 ppm. There was a dose-related increase in lenticular opacities observed in ducklings

administered dinoseb in their diet for a period of 26 days (1000, 300, 200, 100, and 60 ppm) or longer( 60 ppm only), which was more apparent as the study progressed. These effects included central posterior subcapsular haziness of the lens, unilateral or bilateral sutural haziness, and unilateral or bilateral and equatorial lenticular vacuolation(e.l.v., only observed at 60 ppm and in the controls). The effect(e.l.v.) in the 60 ppm ducks was observed prior (by day 26) to a similar phenomenon which was observed in the controls at day 45 but not earlier. This hints at the possibility that dinoseb may have accelerated lenticular changes which may normally occur in ducks. A similar acceleration effect in the mouse (24 month study) has been noted (J. Rowe, 1986; review of mouse oncogenicity study for dinoseb).

Shorter duration (4 day) studies and lower levels were performed to elucidate the time- and dose-response effect for the lower dose levels used(200 and 100 ppm), as well as to establish a NOEL for the study. However, such a short duration for the administration of the compound is not adequate to allow projection of the subchronic/chronic effects of dinoseb. A NOEL for lenticular effects was established at 60 ppm for a four-day regime but one was not established for a longer regime (28 days).

This ancillary study was classified as Core Supplementary.

82-2 Repeated Dose Dermal Toxicity: 21-Day Study

No 21-day dermal toxicity studies with technical Dinoseb were found.

82-3 Subchronic Dermal Toxicity: 90-Day Study

No subchronic dermal toxicity studies with technical Dinoseb were found.

82-4 Subchronic Inhalation Toxicity: 90-Day Study

No subchronic inhalation toxicity studies with technical Dinoseb were found.

82-5 Subchronic Neurotoxicity: 90-Day Study

No subchronic neurotoxicity studies with technical Dinoseb were found.

Due to the dermal toxicity observed in humans (see Section B, Human Toxicity Data), testing for repeated dermal toxicity is required. Consequently, this test now becomes a data gap. The subchronic oral requirement is not considered a data gap, although all reviewed studies were supplementary, since chronic studies are required to support permanent tolerances and preclude the need for subchronic testing. A 90-day dermal and subchronic inhalation and neurotoxicity studies are not required.

IMMUNOTOXICITY

The potential for dinoseb effects on components of the immune system was examined in male hamsters (Dandliker et al.,1980; MRID 114252). Dinoseb was administered orally(corn oil) in a single dose to hamsters 5-8 weeks old at one-half of the $LD_{50}$ value to a group of five animals. Normal delayed type hypersensitivity(DTH) reactions, circulating antibody (serum concentration, heterogeneity) and body weight effects were determined. Dinoseb decreased average body weight in the treated as compared to the controls, at one week following dosing and through the last weighing at five weeks. Antibody titers were depressed in the dinoseb treated group at 21, 35, and 50 days after dosing. The treated group also had a decrease in the number of antibody-combining sites specific for fluorescein (only measured at 50 days after dosing). In addition, dinoseb appeared to depress the average association constant of serum antibody against fluorescein (at 50 days). The pesticide also appeared to interfer with the normal DTH response to ovalbumin.

Due to the lack of individual animal or raw data this study was designated Core Supplementary.

An additional suggestion of the potential immunotoxicity of dinoseb relates to the observation in a mouse oncogenicity study (Brown, 1981; JRDI03) of a treatment-related effect in the thymus. In both sexes, there was an increased percentage of mice showing involution or atrophy of this tissue at all dose levels compared to the controls. The changes appeared to be somewhat more predominant in the females as contrasted to the males.

Additional testing for immunotoxicity concerns are addressed under Toxicological Issues, Section IV.

CHRONIC TOXICITY

83-1 Chronic Toxicity

A 100 week oral (dietary) chronic toxicity/oncogenicity study was performed in CD-1 mice with technical Dinoseb (98% purity) being administered at nominal dose levels of 0, 1, 3, and 10 mg/kg/day (Brown, 1981; JRDI03).

Dinoseb produced an increase in the rate of development of lenticular opacities in both sexes which was noted by 78 weeks in the mid-and high-dose levels but not investigated in the low dose. Also, adverse effects on the reproductive organs of both sexes including the uterus and testes are suggested by the treatment-related lesions observed in this study. In the uterus there was a consistent increase in the incidence of cystic endometrial hyperplasia observed in all treated female groups as compared to the controls. A similar situation was observed in the testes with a report of atrophy/hypospermatogenesis/degeneration and dystrophic calcification in all levels of dosed males which appeared compound-related. This study was designated Core Supplementary. (This study was reviewed mainly as an oncogenicity study and will be discussed further in that section.)

A combined chronic toxicity/oncogenicity study was performed in Charles River rats with the animals being fed Dinoseb in their diets for 104 weeks at nominal dose levels of 0, 1, 3 and 10 mg/kg/day(Piccirillo and Banas, 1977; MRID 25582).

Despite major shortcomings in design and execution of this study, there was no effect on survival, food consumption, or most of the clinical laboratory parameters. There appeared to be a diminution in white blood counts of a somewhat sporadic nature, seen primarily in the male rats. Mean body weights were decreased in males and females receiving the high dose as compared to controls. Gross findings at necropsy were similar among dosed and control animals. In most of the female- and male-groups, mean plasma and erythrocyte cholinesterase activities were slightly lower than respective control values at weeks 13 and 26; however these values were not statistically significant. No histopathological alterations were observed in any of the limited number of tissues examined. Thyroid weights in males were decreased in a dose-related manner with the mean weight being significantly lower than control ($p<0.05$) at all dose levels.

This study had a number of major deficiencies including: the lack of adequate identification of the compound tested; limited histopathologic assessment (10 animals/sex for the control and high-dose groups at the 12-month and terminal sacrifices, and only a few tissues in 10 animals/sex/group of the low- and mid-dose groups; less than a full set of tissues/animal for any group); lack of individual data for body weights for all intervals, animal disposition, and clinical observations; no histopathologic examination for rats that died or were sacrificed in extremis during the study, and only 5/sex/group for clinical chemistry, hematology and urinalysis. For these reasons, this study was classified Core Invalid data and a retest is requested.

Data gaps for chronic toxicity are studies in a rodent and non-rodent, since both of the chronic studies were not acceptable for regulatory purposes.

ONCOGENICITY

83-2 Oncogenicity

A 100 week oral (dietary) chronic toxicity/oncogenicity study was performed in CD-1 mice with technical Dinoseb (98% purity) being administered at nominal dose levels of 0, 1, 3, and 10 mg/kg/day (Brown, 1981; JRDI03).

An equivocal response for oncogenicity was noted in the liver. There was a statistically significant ($p<0.05$), treatment-, but not dose-related increase in liver adenomas and adenomas plus carcinomas in treated female mice when the controls were compared against treated mice. Also reported was a statistically significant ($p<0.05$), treatment-related increase for combined data for these neoplasms (all lesions in both sexes) when compared against the combined control male and female incidences (all lesions). The treated males did not have any statistically significant differences. The study report also noted that in both sexes combined, the incidence for adenoma in treated mice approached statistical significance ($p<0.1$). Since the tumors were noted only in the liver and were benign, the biological significance of the increased incidence is unclear. Other points which argue against oncogenicity are: 1)the lack of a dose response effect, 2) statistical significance in only one sex and 3) no decrease in the latency period for the development of tumors.

This study was designated Core Supplementary with the possibility of being upgraded to Core Minimum if stability data on the stock dinoseb is submitted.

A combined chronic toxicity/oncogenicity study was performed in Charles River rats with the animals being fed Dinoseb in their diets for 104 weeks at nominal dose levels of 0, 1, 3 and 10 mg/kg/day(Piccirillo and Banas, 1977; MRID 25582).

Despite major shortcomings in the design and execution of this study, no no oncogenic response was apparent. However, the oncogenic potential of Dinoseb cannot be adequately evaluated in this study due to major deficiencies including: the lack of adequate identification of the compound tested; limited histopathologic assessment (10 animals/sex for the control and high-dose groups at the 12-month and terminal sacrifices, and only a few tissues in 10 animals/sex/group of the low- and mid-dose groups; less than a full set of tissues/animal for any group); lack of individual data for body weights for all intervals, animal disposition, and clinical observations; no histopathologic examination for rats that died or were sacrificed in extremis during the study, and only 5/sex/group for clinical chemistry, hematology and urinalysis. For these reasons, this study was classified Core Invalid data and a retest is requested.

Data gaps exist for oncogenicity testing in two species; however it is highly probable that the mouse study may be upgraded to Core Minimum and thus fulfill a requirement for one species.

8

005421

REPRODUCTIVE TOXICITY

83-4 Reproduction

In a three-generation reproduction study, 2 littering groups/generation, Sprague Dawley rats were fed dietary levels of 0, 1, 3, and 10 mg/kg/day (nominal levels) of technical dinoseb (98% purity) during the entire period of the study (Irvine, 1981; JRDI11). There were no significant compound-related effects on male and female fertility, the gestation indices, fetal viability or lactation indices. However, there was a consistent, compound-related decrease in body weight gain at the high dose in both adult males and females in the pre-mating period in all three generations, which persisted into later periods. A parental systemic LEL of 10 mg/kg/day, based on depressed weight gain, was established in this study and the NOEL is 3 mg/kg/day. The mean fetal weights were affected by dinoseb administration but with a high degree of variability. Decreased weights were observed or suggested in $F_0$-->$F_{1b}$, $F_1$-->$F_{2a}$, and $F_2$-->$F_{3a}$ littering groups with the $F_0$-->$F_{1b}$ pup weights diminished (combined sexes) at day 21 at all dose levels. Since the treated pup weights at birth were similar to controls, the subsequently decreased pup weight gains indicate a reproductive effect during the lactation period. There were no other significant fetal effects. Based on depressed pup weight gains after birth, a reproductive LEL of 1 mg/kg/day (LDT) is determined and a NOEL was not established. This study is classified Core Supplementary data.

In a continuation for an additional two generations (single litters) of the above study (Irvine, 1981; JRDI11), depressed parental body weights were seen in all treated rats, during one or more periods. There were no significant compound-related effects on any other parental parameters( male and female fertility indices, gestation indices), except for increased ovary weights in the mid level $F_3$ females. Thus, the parental systemic LEL is 1 mg/kg/day (LDT) based on decreased weight gains and a NOEL was not established. The only significant effects on the progeny were those on body weight gains. Weights of $F_{4a}$ pups were increased on day 21 for the low dose group and $F_{5a}$ pups of the low and high dose group had increased weights during lactation. (These increases in body weight are in contrast to the decreases seen during the first three generations.) Testicular weights of $F_{4a}$ progeny at all dose levels were decreased, but the change was not statistically significant. Because of an unacceptably low viability index for $F_{5a}$ controls, the inconsistency of fetal body weight changes between the first 3 generations (above study) and the last two generations (present study), and the consistent depressed gonadal weights of all $F_{4a}$ treated males, it is considered inappropriate to set a NOEL for reproductive toxicity in the pups of this study. The study is classified Core Supplementary data.

Administration of technical dinoseb(97.3% purity at 0, 75, 150, 225, 300 ppm) in the feed of Sherman male rats for an eleven-week period followed by a 16-week recovery period resulted in a dose-related effect on body weight, organ weights, mortality, reproductive performance, fetal viability, sperm number in the testes and epididymis, and sperm morphology (Linder et al., 1982; JRDI14). Male rat body weights were consistently lower than controls during the treatment period at the two highest doses (300>225 ppm: 38 and 19% average weight depression) with partial recovery at the 300 ppm dose after cessation of dinoseb administration and nearly complete recovery at 225 ppm by day 190 or so. An increase in mortality was observed in the high dose group. The males of the 225 and 300 ppm groups were basically infertile when mated at 0-14 days post-treatment.

Remating at 104-112 days post-treatment did not result in any substantial increase in fertility. Statistically significant decreases in the weight of the testes and epididymis were observed at 225 and 300 ppm dose groups as compared with controls at 11 weeks. After a 16 week recovery period, depressed body and organ weights of the two highest dose groups had not completely recovered.

The sperm counts were significantly reduced at 150, 225, and 300 ppm dose levels. After a 16 week recovery period the 150 ppm dose group had a normal count; the 225 dose group had partial recovery for the sperm count, and the high dose group sperm count was unchanged. By day 20 of treatment only 10% of the epididymal sperm cells in the 300 ppm dose group were normal looking, and animals sacrificed subsequent to 50 days of treatment had oligospermia. Many spermatozoa were atypical in the 225 and 150 ppm dose groups at the 11th week sacrifice, with the rats (3/5) fed 225 still having abnormal profiles after a 16-week recovery period.

This study was classified as Core Supplementary.

Spencer and Sing (; JRDI23) reported on the reproductive toxicity of technical dinoseb (95.0% purity) in day 10 pseudopregnant and day 16 pregnant rats fed dinoseb in their diet from days 6 through 9 of pseudopregnancy at 25 to 750 ppm or for pregnant rats fed from day 6 through 16 of gestation at 50 to 350 ppm. Their findings suggest that dinoseb's reproductive toxicity (decreased fetal survival at birth, decreased fetal weight) may be mediated through an effect on the uterine physiology, i.e., the ability of the uterus to adequately support normal fetal development. However, it should be noted that the fetal effects are noted at higher doses ($\geq$150 to 200 ppm) in the pregnant animals as compared to uterine changes (decreased weight, glycogen content) in the pseudo-pregnant rats which occur at lower doses (25, 50, 100 ppm). This study was classified as Core Supplementary for the reasons stated in the study review.

In summary, review of two reproductive studies (3 generation/two litters per generation; 2 generation/one litter per generation) and two special reproductive studies (testicular effects and mechanism of dinoseb reproductive toxicity in the uterus), all of which were classified as Core Supplementary, indicates a data gap for reproductive toxicity.

005421

ERATOLOGY

83-3 Teratogenicity

In the caesarian data from a 3 generation study (Irvine, 1981; JRDI11), ariability in the response of rat pups to the fetotoxic effects of Dinoseb were observed. In $F_0(F_{1b})$ pups there was an apparent dose-related increase in overll skeletal defects ("minor" fetal defects), as compared with the control which as statistically significant at the high dose (10.1%=cont., 14.3%=low, 23.3%=mid, 3.8% = high [% of fetuses examined]). For the $F_2(F_{3b})$ pups, there was an apparent ompound-related increase (not statistically significant) in the total number of minor" skeletal defects, due primarily to an increase (treatment-related) in sterebral and rib defects, while variants (forelimb or hindlimb phalanges incompletely/ ot ossified) were consistently increased in a compound-related fashion. However, the 2b pups did not appear to show any dose- or compound-related effects. A NOEL annot be established in this study because: 1) the small number of dams utilized 9 to 10) precludes the determination of fetotoxicity with any statistical confience, 2) the investigators did not present litter incidence for fetal defects, and ) finally, the pre-implantation loss is quite variable in the controls, making nterpretation of the findings uncertain. The study was classified Core Supplentary.

Gibson (1973; MRID 57711, 39868) studied the teratogenic effects of dinoseb dministered daily to Swiss-Webster mice by the intraperitoneal(ip), subcutaneous sc) or oral route. The dinoseb was administered at various periods of organogensis: days 8-16, days 10-12 and days 14-16 of gestation.

IP injection produced dose-related teratogenic/variation effects when adminstered during 10-12 days of gestation with a significant increase in internal /drocephalus at 10 mg/kg/day and numerous significant external (oligodactyly, perforate anus, acaudia, microcaudia, amelia), soft-tissue (internal hydrocephalus, hydronephrosis) and skeletal(fused and absent ribs, fused/absent or not ossified sternebrae, fused/not ossified/absent vertebrae, and absent or not ossified ong bones) anomalies at a dose of 17.7 mg/kg/day. Sc injection was reported to oduce cleft palate (10%) at the high dose only, with statistically significant ncreases in anomalies when administered at days 10-12 (fused ribs, absent or not ssified sternebrae and fused vertebrae) and days 8-16 of gestation (supernumerary ribs, absent or not ossified sternebrae and supernumerary vertebrae) but ot during days 14-16 of gestation. Oral intubation produced a significant increase in absent or not ossified sternebrae when administered during days 14-16 f organogenesis, while a dose-related increase occurred at 20 and 32 mg/kg/day osages for supernumerary ribs and an increase at 32 mg/kg/day for supernumerary ertebrae when administered during days 8-16 of gestation. Embryotoxicity (decreased c-r length) was observed only at 32 mg/kg/day. A NOEL can not be established with any confidence due to the small number of animals tested. This study as not designed for regulatory purposes and was designated Core Supplementary ata.

Based on review of the studies discussed above, and several teratology creening studies, all of which have been classified as Supplementary data, (see aragraph below) data gaps for teratogenicity studies in two species exist.

There are a number of additional teratology studies reported in the open
erature which are basically of a screening nature and are listed below in a
le. All of these studies are classified as Core Supplementary due to the mode
compound administration, time of compound administration during organogenesis,
k of individual animal data, and so forth. These studies suggest a fetotoxic
ential of dinoseb both pre-natally and post-natally, as well as the importance
environmental factors, and the route of administration in chemically-induced
atogenic or fetotoxic responses to Dinoseb.

005421

TERATOLOGY "SCREENS"

| Type of study/ study i.c. | Dosage regimen | Study Findings |
|---|---|---|
| 1. ip injection in female mice/ Preach, Gibson, 1975(JRDI19) | 0, 14.1, 15.8 mg/kg/d on days 10-12 gestation; 17.7 mg/kg/d on day 11 gestation; 18.8 mg/kg on day 12 gest.; 15.8 or 17.7 mg/kg/d on day 12 gest. (1 hr after pretreatment with SKF-525A); 15.8 mg/kg/d after 3 days pretreatment with phenobarbital (50 mg/kg; twice daily) | Dinoseb produced major teratogenic defects; after animals were fasted for 24 hrs compound's effect magnified but not after 48 hr; pretreatment with phenobarbital inhibited the teratogenic/tetotoxic effects from 24 hr fast, while pretreat- with SKF-525A enhanced the effect; these effects appear to be mediated thru alterations in in vivo metabolism |
| 2. oral intubation in female mice/Chernoff, Kavlock,1982 (JRDI04) | 15 mg/kg (MTD) on d 8-12 of gestation | Dinoseb did not give evidence of teratogenicity/fetotoxicity as measured by effects on litter size, maternal wt. changes, pup viability or pup wt. changes(day 1, 3 after birth) |
| 3. oral intubation in female mice/Gray, Kavlock,1984 (JRDI09) | 0, 15, 100 mg/kg/d during days 8-12 of gestation | No significant effects on postnatal parameters including number of pups alive d 3, b.wt at d 3, b. wts. at day 22 or 30 (both sexes) or day 57 (males), and no decreases in body or organ wts at day 250 |
| 4. oral intubation in female mice/Kavlock et al.,1985 (JRDI13) | 0, 26, 33 mg/kg | Significant decrease in enlarged renal pelves at both doses and 50% increase in supernumerary ribs(stat. signif.)at both dose levels, which was inversely related to maternal weight |
| 5. ip injection in female rats/ McCormack et al., 1980(JRDI15) | 0, 6.3, 9.0, 11.2, 12.5 and 15.0 mg/kg during d 10-12 gestation | 8.0 and 9.0 mg/kg-resulted in postpartum effects:decreased fetal b.wt. (d 1&7,not d 42), histological chang in liver[vacuolations,necrotic(d 42) and kidneys(dilation of renal pevis and tubules, vacuolation of transitional epithel. of ureters); these effects in kidney diminished by d 42 |
| 6. ip injection in female rats, in vitro culture/ Beaudoin, Fisher, 1981 (JRDI02) | 10 mg/kg at day 9-10 of gestation; embryos cultured up to 42 hrs | Significant inhibition in the development of embryonic axis and neural tube closure in cultured embryos (both at 24 hrs, 42 hrs after removal from dam) |

1

MUTAGENICITY

84-2 Mutagenicity

1) Gene mutation

a. Bacterial studies

Simmon et al.(1977; MRID 132582, 5009139, 24901) evaluated dinoseb in S9-activated and nonactivated plate incorporation assays (1-1000ug/plate) with S.typhimurium TA100, TA1535, TA1537, TA1538 and E. coli WP2. Dinoseb was cytotoxic at the high dose for all strains (±S9). No increase in either histidine or trytophan reversion, respectively, was observed for any strain with or without activation. The study is considered acceptable since dinoseb was assayed up to cytotoxic doses.

Shirasu et al. (1982; JRDI20) conducted a S.typhimurium and E. coli mammalian microsome assay with unreported doses of dinoseb. The information was insufficient to draw meaningful conclusions, and was designated unacceptable.

In an Ames test (S. typhimurium, TA98, TA100, TA1537, TA1538; ±S9), Eisenbeis et al.(1981; JRDI08) found that dinoseb concentrate, the commercially used concentration(which was not reported), and dinoseb in combination with other herbicides( not stated) were negative. The study was acceptable.

No increase in histidine revertants of S. typhimurium TA100, TA98, TA1537, and 1538 or trytophan revertants of E. coli WP2 was observed after exposure to 50, 500 and 5000 ug/plate of dinoseb (±S9) (Moriya et al., 1983; JRDI16). Although the study did not include a positive control, it was considered acceptable since dinoseb was tested at a high dose (5000 ug/plate) and the ability to detect an effect was evident in the positive response of 50/228 pesticides tested.

Another Ames test(8 histidine-deficient strains; 1-5ul spotted) was considered unacceptable since it was conducted without S9 activation (MRID 5001460).

b. Fungi

Gillberg (1971; MRID 500588) failed to isolate dinoseb-resistant Rhizobium mutants following exposure of auxotrophic fungal strains to 50, 100 and 150 ug/ml of dinoseb. The study provided no meaningful data and was judged unacceptable.

c. Insects

Valencia (cited in Waters et al., 1980; JRDI24) investigated 0.5 and 1-4 ppm dinoseb in a sex-linked recessive lethal Drosophilia melanogaster assay. The author reported that the compound had not been tested adequately due to severe toxicity and the very low concentrations evaluated. This study was considered unacceptable.

2) Structural chromosomal aberration tests

None found.

14

3) Other genotoxic tests

a. Primary DNA damage

Procaryotes:

Simmon et al. (1977; MRID 132582, MRID 5009139, MRID 24901) reported on a properly controlled study with one mg/disc of dinoseb (-S9) which caused preferential inhibition of DNA repair-deficient E. coli p3478 and B. subtilis M45. The studies were positive for primary DNA damage(not performed with S9 activation) and are considered acceptable(-S9).

Waters et al. (1982; JRDI25) evaluated the effects of dinoseb in a S. typhimurium SL 4525 (rec+)/Sl 4700(rec-) differential toxicity assay. Dinoseb produced preferential inhibition but the study did not provide the dose at which the compound was active and therefore the results were viewed only as qualitative in nature. The study is unacceptable.

Using E. coli C600, W1665F (lambda and M12 phages) a prophage induction test (Toure and Stenz, 1977: MRID 5016148) with nonactivated dinoseb at $10^{-2}$ to $10^{-9}$ M provided no meaningful data. This study is unacceptable.

Eucaryotes

Two independent S. cerevisiae D3 mitotic recombination assays were performed with dinoseb (+S9) with 0.3%, 0.2% and 0.1% concentrations( Simmon et al., 1977; MRID 132582, MRID 5009139, MRID 24901). In the first assay, a six-fold increase in both the absolute number of mitotic recombinants/ml and the relative number of recombinants/$10^5$ survivors was observed (0.2% nonactivated dose) while in the second assay, $\geq$ threefold increases in both the absolute and relative number of mitotic recombinants were reported (+S9) for 0.2% dinoseb. The results suggest weak genotoxicity and the study is considered inconclusive.

Siebert and Lemperle (1974; JRDI21) exposed late logarithmic phase S. cerevisiae D4 for 16 hours to 100 and 1000 ppm of dinoseb (-S9). A slight effect was noted at the low dose. This study was not considered appropriate for evaluating the test material and was an unacceptable study.

Mammalian cells

Simmon et al (1977; MRID 132582, MRID 5009139, MRID 24901) reported on the exposure of human diploid fibroblast WI-38 cells to $10^{-4}$ to $10^{-7}$ M dinoseb for 3 hours (-S9) and to $10^{-3}$ to $10^{-5}$ M dinoseb (1-hr treatment; +S9). Using liquid scintillation counting of unscheduled DNA synthesis, genotoxity was not apparent. The study was acceptable.

b. Genotoxicity in germinal tissues

Osterloh et al. (1983; JRDI17), in a mouse sperm morphology study, administered to male mice five daily gavage doses of 2, 4.3, 9.3, 20 and 43 mg/kg of dinoseb. This study provided no useful data, possibly due to the route of administration or the selected sampling interval, for detection of mutagenic effects on early and/or late stages in the cell cycle. The study was designated unacceptable.

In summary, Dinoseb has been found:

1. for gene mutation, negative in three bacterial studies which have been classified acceptable but no mammalian cell assays were submitted;

2. for DNA damage,

a) positive in procaryotes (without S-9 activation) in a study classified acceptable,

b) weakly positive in eucaryotes in a study classified inconclusive, and

c) negative in mammalian cells (human fibroblasts) in a study classified acceptable.

An in vitro mammalian cell assay for gene mutation (i.e., mouse lymphoma, Chinese hamster ovary cell/HGPRT, or V79 Chinese hamster cells) and a structural chromosomal aberration test are mutagenicity data gaps.

METABOLISM

85-1 Metabolism

The disposition of radiolabeled dinoseb in pregnant mice was studied by Gibson and Rao (1973, MRID 39869). They found the route of administration to be critical in the absorption of dinoseb, i.p. injection giving a plasma peak of radioactivity at approximately 8 minutes in comparison to 12 hours when administered via oral intubation. Dinoseb is eliminated roughly 4X faster after ip compared to oral administration, through biliary excretion and subsequently in the feces. Differential metabolism in the gastrointestinal tract(intestinal flora) for oral versus ip administration may account, in part, for the teratogenic effects observed at lower doses with ip injection. Metabolism has been reported in the rabbit, rat and various ruminants. Rabbits, but not rats, reduced one nitro group of dinoseb with the formation of the 6-amino derivative conjugated as the o-glucoside. A carboxylic acid was also formed by oxidation of the terminal carbon of the secondary side chain in both rats and rabbits. In rumen fluid, dinoseb (under anerobic conditions) was converted to the 6-amino derivative, with successive reduction to the diamino compound. This reduction did not take place in heat sterilized rumina. In vivo, the diamino compounds were not demonstrated in blood plasma in the cow. Conversion of parent compound to labeled metabolites (%) in the pregnant mice appeared to be roughly equal via both oral and ip routes (50%). The one exception was the embryo where the radioactivity of the metabolites recovered was higher for the ip injected animals (57%) as opposed to the orally--treated mice (90%). This would suggest that the unknown metabolites in the embryo might account, in part, for the differential effects seen. The study is Core Supplementary data.

The effect of food deprivation, phenobarbital, and SKF-525A on dinoseb-induced teratogenicity in female mice and on its biodisposition ([$^{14}$C]dinoseb) was studied by Preache and Gibson (1975; JRDT19). Mice were dosed on day 10, 11 of gestation or after 1-hr pretreatment with SKF-525A or after 3 days' pretreatment with phenobarbital or after 0, 24, or 48 hrs of food deprivation. It was concluded that alteration of the environmental conditions (food deprivation for 24 or 48 hrs) either enhances or has no effect on the teratogenic or fetotoxic effects of dinoseb administered ip to Swiss-Webster mice during days 10-12 of gestation. This appears to occur through a decrease in the metabolic inactivation of dinoseb with 24 hrs of food deprivation and an increase in metabolism after 48 hrs of food deprivation as evidenced by a reduction in the rate of disappearance of plasma radioactivity or an increased rate of disappearance of radioactivity from the liver, respectively. Administration of SKF-525A also enhances the toxicity of dinoseb while phenobarbital decreased it. These effects are mediated through either an inhibition of metabolism of the dinoseb or an enhanced metabolic effect in the liver. Evidence in nonpregnant mice supports these findings. The altered embryotoxicity from food deprivation is not apparently due to an altered volume of distribution since food deprivation had no effect on the percent body water determined under fasting conditions. The study is Core Supplementary data.

The effects in female mice of high and low environmental temperatures on maternal and fetal toxicity of dinoseb and on disposition of [$^{14}$C]dinoseb were reported by Preache and Gibson (1975; JRDT18). There were no differences in the rate of disappearance of radioactivity from plasma, liver, kidney and lung for mice kept at 0, 24 or 32°C. The authors had shown, in the same report, that

increased embryotoxicity would occur if the body temperature of the dams were elevated to 32°C. Based on their findings with nonpregnant females, they suggested that the increased embryotoxicity was not related to an inhibition in the rate of disappearance since there were no differences in this parameter for mice kept at 24 or 32°C. They also noted that, while caution was necessary in extending the pharmacokinetic results from the nonpregnant to the pregnant situation, a previous study (Gibson and Rao, Fd. Cosmet. Tox. 11:45-52, 1973) had shown that the disappearance of dinoseb from the plasma of pregnant and nonpregnant female mice was not different. This study was Core Supplementary data.

In a letter to the editor, Henneberg (1964; MRID 60767) reported on the absorption curves(UV) of metabolites found in the livers of rats poisoned with dinoseb or from incubation of supernatants of liver where dinoseb (DNBP) was added (in vitro). The authors concluded that the primary amines are the products of the metabolism DNBP and that the enzymatic reduction process of this herbicide mainly occurs in the liver. This study was classified as Core Supplementary.

Ernst and Baer (1964; MRID 33636, MRID 60766) studied the metabolism of dinoseb and its esters (DNBP-acetate, DNBP-1,1-dimethylacrylate) in rabbits and rats following single oral doses. They observed that free DNBP, its acetic acid and 1,1-dimethylacrylic acid are transformed in the same way in the rabbit and the rat: side chain oxidation and 1-$NO_2$ reduction with hydrolysis of the ester. Reduction products (amino compounds) of DNBP or its esters could not be demonstrated in the urine of rats but were present in rabbit urine as 2-sec-butylamino-4,6-dinitrophenol, as well as up to 60% of the total excretion products as an o-glucuronide of this compound. This may be due to formation of the glucuronide through a reduction of a nitro group in the rabbit, which does not occur in the rats due to inhibition of the nitro group. Excretion of the parent compounds was greatest during the first 48 hours after administration of DNBP or its esters. By day 10, around 1% of the amount excreted during the first 2 days was still being excreted. These data were designated Core Supplementary.

Edgerton and Moseman (1978; JRDI06) analyzed feed, fat, liver, blood, urine and feces after giving rats diets containing 50 and 200 ppm of dinoseb. Blood was the major tissue site for dinoseb concentration. There was a proportional increase (4x) in dinoseb residues in adipose tissue and liver with blood exhibiting a three-fold increase when the dinoseb concentration in the feed was increased from 40 (50 nominal) to 160 (200 nominal) ppm. This study is Core Supplementary data.

A data gap exists for a general metabolism study conducted according to current guidelines.

B. HUMAN TOXICITY

1. Acute effects

Dinoseb(DNBP) has pharmacological and toxicological actions related to the extreme stimulation of metabolism due to the dissociation of carbohydrate oxidation and phosphorylation at the cellular level(Smith, 1981; JRDI22). This uncoupling results in general cellular over-activity producing widespread metabolic upset associated with fatigue, excessive sweating, thirst and loss of weight. Dinitro compounds, or their metabolites, can cause some degree of hepatic and renal damage and produce neurotoxic symptoms varying from mild personality changes to a toxic psychosis with convulsions. Elimination of dinoseb turns the feces and urine a bright yellow. Also, the skin appears very active in the excretion of dinitrophenols and yellow staining of the skin and hair and nails is commonly seen in severe poisoning.

Smith (1981; JRDI22) has reported on the suspected poisoning of a self-employed farmer from the spraying of a contact herbicide containing dinoseb. The farmer repaired a clogged jet on his sprayer (while it was running) without any protection for his hands. Initial symptoms of poisoning were headache, malaise, lassitude and sweating, and yellow staining of his fingers. During the next week the patient experienced anorexia, bouts of excessive sweating and shivering, pains in the abdomen, excessive thirst, restlessness, insomnia, loss of weight (10kg) and generalized yellow staining of the skin and sclera. He also developed respiratory symptoms such as shortness of breath and hemoptysis and personality changes. Admission to a hospital revealed flushing, dyspnea, spasmodic coughing, dullness at the base of the lung, and crepitations. The urine was discolored yellow. Liver function was impaired and lung function tests indicated a considerable reduction in forced expiratory volume in one second ($FEV_1$-2.51) and forced vital capacity(FVC-3.51) as compared to predicted values of 4.1±.5 and 5.1±.58, respectively. The transfer factor was 22.1 ml/mm Hg, the normal being 20 (s.d. 5.1). At the end of one week in the hospital, his clinical condition had improved but at discharge his liver function tests remained abnormal. Some two weeks later, he still complained of lethargy, night sweats and forgetfulness. By 10 to 12 weeks the farmer had no symptoms but at six months his blood urea was elevated (7.9 mmol/l, normal range 3.5-6.5 mmol/l).

A human fatality from dermal exposure to dinoseb (51% a.i. dinoseb as alkanolamine salts of ethanol and isopropanol series) during its use in an agricultural setting in Texas has been reported (Memo re: information on farmworker death; Sept 28, 1983; N. Dyer to D. Campt; includes autopsy report from Texas Dept. Agr.: PA-83-110). A farmworker, who was an illegal alien, was exposed from leakage onto his back from a backpack sprayer. Dermal exposure through the hands and feet also apparently occurred since yellow stains were observed on these skin areas. The man experienced toxic symptoms typical of dinitrophenol poisoning including fatigue and headache during the afternoon and evening on the day of exposure. The worker was hospitalized that evening and died within a half-hour of his admission.

DNOC (2-methyl-4,6-dinitrophenol), a close analog of DNBP, was reported to produce eight occupational deaths during the period of 1946-1951 in Britain among pesticide spraying contractors' employees (Edson, 1969; JRDI07). DNP (dinitrophenol) itself has been reported to be involved in 40 accidental poisonings during the period of 1966 to October 1980 (Pesticide Monitoring System; Report

No. 384) including one human fatality (11 year-old boy who died after he apparently was sprayed with pesticide in an undescribed agricultural job-related incident).

In response to an inquiry by J. Ward of the Pesticides Regulation Division (August 16, 1965), the Dow Chemical Company submitted occupational information regarding the medical experience of their employees with dinoseb (Lynn, 1965; MRID 90368). A letter was submitted which indicated that their Medical Department had maintained surveillance over workers in the dinitro manufacturing operations since 1944. Examination of the workers for basal metabolic rates (BMRs), blood studies and cataract formation (1954, 1955) was reported as within normal limits except for an episode in February, 1956 of over-exposure, apparently to DNOC, which produced symptoms(kind not stated) and high BMRs in several men. No additional ill effects were reported by plant physicians in the dinitrophenol operation since 1956.

In a followup submission to the previous letter, BMR and time-weighted average exposure (TWA) data were submitted to J. Ward (Lynn, 1966/MRID 90300; Dow Chemical Co., 1956/MRID 114965). (The value of the data is questionable since average BMR values for workers were not determined prior to the time the workers were believed to be exposed nor at the time of exposure). The maximum BMR value considered by the registrant as normal was stated as +10%. Review of the data (single page of information) indicated elevated BMR values for several men during the testing period of 7/20/54 through 5/28/56, including values of +48, +18, +46, +25, +11, +59, +33, +13, +27, +40, +17 (represents individual workers). TWA values for the same time period (during production, annual) were given as well as blood concentrations(ug/ml) from January, 1956 on. TWA values ranged from 1.0 to 5.7 mg/man/day during production and from 0.14 to 3.2 mg/man/day annually. Blood values ranged from 1.2 to 9.2 ug/ml. The exposures appeared to diminish somewhat after January, 1956, although the representative nature of these data is unknown.

2. Ocular effects

A report was submitted by Dow Chemical Co. regarding the results of ocular examination of workers employed in the manufacture and handling of dinitrophenols (Gay, 1951; MRID 67701, 90369). The report indicated that 28 men were exposed to DNBP and dinitro-o-cyclohexylphenol almost continuously for 5 years during their work period. Exposures were sufficient to cause yellow staining of the clothing, the skin and hair. No ill effects were reported. Fifteen of 18 men who were employed at that time in those operations(3 with 5 years' exposure, 2 with 15 to 18 months' exposure, and 10 with 2 to 9 months' exposure) were examined by ophthalmologists who reported no ocular abnormalities attributable to chemical exposure. Four other ex-employees (20-39 months' exposure) were also reported to have no ocular effects . No individual data were submitted in this report.

II. DATA GAPS

Dinoseb (2-sec-butyl-4,6-dinitrophenol) is registered for use as a nonselective herbicide for preemergent and postemergent weed and grass control in agricultural food crops, and noncrop areas, and as a preharvest desiccant in various seed crops. Therefore, the following routine or special Guideline toxicology studies are required for registration( those which currently are data gaps are indicated by an asterisk):

Acute testing

1. acute oral toxicity
* 2. acute dermal toxicity
* 3. acute inhalation toxicity
* 4. primary eye irritation
* 5. primary dermal irritation
* 6. dermal sensitization

Subchronic testing

* 1. 21-day dermal toxicity

Chronic testing

* 1. chronic feeding-2 species: rodent and non-rodent
*? 2. oncogencity-2 species: rat and mouse preferred
* 3. teratogenicity-2 species
* 4. reproduction-2 generation

Mutagenicity testing

* 1. gene mutation: bacterial tests acceptable; mammalian test required
* 2. structural chromosomal aberration
3. other genotoxic effects

Special testing

* 1. general metabolism
* 2. testicular toxicity

Based on an assessment of the toxicology data base for dinoseb, completed in this Standard, all of the above studies which have an asterisk are currently data gaps. It is highly likely that the submitted mouse oncogenicity study may be upgraded to Core Minimum classification and thus fulfill the requirement for an oncogenicity test in one species. It is the reviewer's understanding that teratology studies have been conducted in both the rabbit and rat, and the rabbit study has been received by the Agency (letter of Dec. 11, 1985 from D. Lawatsch, American Hoechst Corp. to R. Mountfort, EPA; J. Stone, personal communication). These studies may fulfill the requirement for teratogenicity testing. The rabbit study will be reviewed in an expedited manner and included as an addendum to the Dinoseb Registration Standard. An additional special requirement for dinoseb is the need for a 21-day dermal toxicity study. Justification for this requirement

is based upon accident reports of human fatality/morbidity incurred subsequent to dermal exposure. Finally, a special reproductive study for testicular effects is required based on the findings of testicular atrophy in rats exposed to Dinoseb as reported by Linder et al. (1982; JRDI14). This last test could be incorporated into the protocol for a 2-generation reproduction test.

## III. TOLERANCES AND TOLERANCE REASSESSMENT

The original PADI of 0.0013 mg/kg and published tolerances are based on a ninety day dog study (unidentified in the files) with a NOEL of 100 ppm (2.5 mg/day) and a safety factor of 2000 ppm (memo of G. Burin to H. Jamerson, 10/12/84). A copy of the approved permanent tolerances is presented in Appendix V. Review of the Dinoseb data base for this Registration Standard indicates that, while there are no studies with at least a Core minimum classification which would allow the setting of a new ADI or PADI, there are two Core Supplementary studies: a chronic mouse feeding study (Brown, 1981; JRDI03) and a rat 3-generation reproductive study, which are appropriate for setting a provisional limiting dose(PLD). Since both studies utilized the same dose levels (0, 1, 3, 10 mg/kg/day) and the chronic study was reviewed primarily for oncogenicity, the LOEL of 1 mg/kg/day set in the reproductive study is deemed more appropriate for use in the PLD calculation.

Calculation of the PLD:

Using the LOEL of 1 mg/kg/day for reproductive toxicity and a 1000-fold safety factor, due to the lack of key acceptable studies, and an additional 3-fold factor because there is a LOEL and not a NOEL, a PLD of 0.00033 mg/kg/day is determined. The MPI (maximal permissible intake) for a 60 kg human would be 0.0198 mg/day.

Published and unpublished approved tolerances currently utilize 71.36 % of the PADI of 0.0013 mg/kg/day. The tolerance reassessment, by requiring the substitution of a PLD for the current PADI, will result in the utilization by the currently approved tolerances of 270.3% of the PLD. It is recommended that no further new uses or tolerances be granted until all data required in the Standard are submitted and evaluated, and all toxicological issues, e.g., teratogenicity, reproductive/testicular effects, presence of nitrosamines and lenticular opacities, have been resolved.

## IV. TOXICOLOGICAL ISSUES

### A. Acute toxicity

Dinoseb is hazardous to humans and animals as evidenced by the reports on accidental poisonings and fatalities (see Section B. Human Toxicity), as well as its placement in Category I because of its acute oral toxicity. Therefore consideration should be given to providing sufficient protection from accidental spills of the pesticide onto the skin or excessive exposure by inhalation during its use.

### B. Immunotoxicity

As noted in the toxicological summary and study reviews, there is some suggestion that immune system components may be adversely affected by exposure to dinoseb. In studies in hamsters, dinoseb appears to interfere with the development of normal delayed type hypersensitivity response and may cause some suppression of circulating antibody in test animals( Dandliker et al., 1980; MRID 134602). Another suggestion of immune-related toxicity is the observation in a mouse oncogenicity feeding study of a consistent, treatment-related increase over control incidence of thymus atrophy (Brown, 1981; JRDI03) at all dose levels. In both sexes, there was an involution or atrophy of this tissue which appeared to be somewhat predominant in the females as contrasted to the males. Effects of dinoseb on the human immune system are unknown.

On the basis of these preliminary observations, it is recommended that during subchronic and chronic testing special attention be paid to the thymus, liver, spleen, bone marrow and cellular components of the immune system, e.g., eosinophils, circulating lymphocytes, monocytes, etc. If there are observations of dose- and/or treatment-related effects on organ, organ-to-body weight ratios, or other parameters, then consideration should be given to the appropriate histopathological preservation of these tissues. Consideration should be given to periodic measurement of circulating serum immunoglobulins.

### C. Lenticular opacities (cataracts)

Dinitrophenols have been associated with lenticular opacities(l.o., cataracts) in humans since their use in the 1930's as weight reducers and are cataractogenic in ducklings and young rabbits ( Hayes, 1982; JRDI26). Lenticular opacities have been reported in repeated-dose studies (a few days up to 28 days) in Pekin ducklings (Spencer et al., 1948: MRID 90374; Tucker and Bennett, 1967: MRID 114963), in a 90-day study in beagles (McCollister et al., 1967:MRID 114962), and in a mouse 2-year feeding study (Brown, 1981; JRDI03).

No special testing is required for a cataractogenic effect since it has been demonstrated already in several species. One of the data gaps requires a chronic toxicity test in a nonrodent; usually the dog is utilized. Chronic testing routinely includes an ophthalmological examination prior to test administration and at termination of the study in at least the high dose and control groups. This examination should be expanded to include <u>all</u> dose groups and an interim examination of the dogs prior to study termination. If cataracts are observed, then gross and histopathological examination of the eyes of all animals should be performed, with special emphasis placed on examination for this effect.

D. Teratogenicity

Dinoseb has been shown to produce teratogenic/embryotoxic effects following intraperitoneal and subcutaneous injection or oral intubation in mice (Gibson, 1973: MRID 57711; Preache and Gibson, 1975: JRDI19; Kavlock et al., 1985: JRDI13) and in in vitro rat embryos cultures (Beaudoin and Fisher, 1981: JRDI02). Postpartum effects such as decreased fetal body weights, and histological changes in the liver and kidney of rat pups have also been observed (McCormack et al, 1980; JRDI15). No acceptable teratology studies are available for the establishment of a NOEL for teratogenic/embryotoxic effects at this time. However, it is the reviewer's understanding that teratology studies have been conducted in both the rabbit and rat and the rabbit study has been forwarded to the Agency (letter of Dec.11, 1985 from D. Lawatsch, American Hoechst Corp. to R. Mountfort, EPA; J. Stone, personal communication). An expedited review of the rabbit study will be performed and included as an addendum to the Registration Standard.

E. Oncogenicity

An equivocal response for oncogenicity was noted in the liver of mice (Brown, 1981; JRDI03). It was reported that a statistically significant ($p<0.05$), treatment-, but not dose-related increase in liver adenomas and adenomas plus carcinomas was found in treated female mice when the controls were compared against treated mice. Also reported was a statistically significant ($p<0.05$), treatment-related increase for combined data for these neoplasms (all lesions in both sexes) when compared against the combined control male and female incidences (all lesions). The treated males did not have any statistically significant differences. The study report also noted that in both sexes combined, the incidence for adenoma in treated mice approached statistical significance ($p<0.1$). An additional statistical analysis by the Toxicology Branch Mission Support Staff(MSS) supports the study author's evaluation (see D.E.R. for the mouse oncogenicity study) of the statistical significance of the liver adenomas in the female mice. Historical data supplied by the test laboratory were also evaluated and did not change the basic conclusions of the study report for oncogenicity in female mice liver adenomas.

Since the tumors were noted only in the liver and were benign, the biological significance of the increased incidence is unclear. Other points which argue against oncogenicity are: 1)the lack of a dose response effect, 2) statistical significance in only one sex and 3) no decrease in the latency period for the development of tumors.

This study was classified as Core Supplementary data. It should be noted that while a rat chronic feeding study failed to show an oncogenic effect, there were significant deficiencies in the study, including the lack of complete histopathological data, which made the study Core Invalid data and which precluded an adequate evaluation of Dinoseb's oncogenic potential in the rat (Piccirillo and Manas, 1977; MRID 25582).

No additional recommendations are made at this time. The oncogenicity of Dinoseb has been considered in an abbreviated review by the Toxicology Branch Peer Review Committee(see Appendix VI). The Committee tentatively concluded that Dinoseb is a Class C oncogen based on the positive liver tumor incidence in female mice. No risk assessment is recommended at this time.

F. Nitrosamines

Formulations containing the alkanolamine salts of Dinoseb may present an oncogenic hazard due to the presence of N-nitrosamines, primarily N-nitrosodiethanolamine (NDELA), which has been reported at concentrations of 200 to 300 ppm in certain products( MRID 25579; MRID 70514). Apparently, this is a potential hazard related only to formulations with alkanolamine salts (memo of Sept. 22, 1983 from Bradley to H. Jamerson) and the use of alternate formulations will eliminate the possible exposure to high levels in such formulations.

G. Testicular/Reproductive Effects

As discussed under the reproductive summmary section, dinoseb(0, 75, 150, 225, 300 ppm; administered in the diet for an eleven-week period followed by a 10-week recovery period) resulted in dose-related effects in male rats of diminished body weight gains, decreased organ weights, mortality, decreased reproductive performance and fetal viability, decreased sperm number in the testes and epididymis, and altered sperm morphology (Linder et al., 1982; JRDI14). Based on the findings of testicular toxicity and the lack of a NOEL in a multi-generation reproduction study, reproductive toxicity testing, which includes testicular effects, should be performed.

H. Structure-Activity Relationships (SAR)

Dinoseb is structurally similar to dinitrophenol (DNP) and a number of other DNP analogs which have a similar metabolic action of stimulation of oxygen consumption through an inhibition of oxidative phosphorylation (Hayes, 1982; JRDI26). A summary table of toxicological endpoints is presented below.

Dinoseb along with DNP and dinitro-o-cresol(DNOC) are quite toxic being in category I or II for acute oral toxicity. Due to their larger side chains, which reduce their rate of absorption, 2-sec-butyl-4,6-dinitrophenyl-3-methylcrotonate (binapacryl) and dinitrooctyl phenol(dinocap) have lower acute toxicities, although it should be noted that the intravenous $LD_{50}$ in male rats for Dinocap is 5 mg/kg as compared to a $LD_{50}$ of 40 mg/kg for Dinoseb in rats (oral intubation).

Generally speaking, the DNP analogs are reported as cataractogenic in humans and a number of animal species, including ducklings (a consistent observation), chickens, dogs, mice and rabbits. Interestingly, rats appear to be refractory to the cataractogenic effects of the dinitrophenols.

Another consistent findings is the observation of reproductive/testicular effects for Dinoseb, dinitrophenol and other analogs. Although binapacryl did not show any reproductive toxicity the dosage tested was relatively low. The SAR data also suggests that dinitrophenol analogs may be teratogenic, although test results have only been reported for two analogs, Dinocap and Dinoseb, at the present time.

Findings related to oncogenicity and mutagenicity are inconclusive. Additional testing is required to clarify these issues.

F. Nitrosamines

Formulations containing the alkanolamine salts of Dinoseb may present an oncogenic hazard due to the presence of N-nitrosamines, primarily N-nitrosodiethanolamine (NDELA), which has been reported at concentrations of 200 to 300 ppm in certain products( MRID 25579; MRID 70514). Apparently, this is a potential hazard related only to formulations with alkanolamine salts (memo of Sept. 22, 1983 from M. Bradley to H. Jamerson) and the use of alternate formulations will eliminate the possible exposure to high levels in such formulations.

G. Testicular/Reproductive Effects

As discussed under the reproductive summmary section, dinoseb(0, 75, 150, 225, 300 ppm; administered in the diet for an eleven-week period followed by a 16-week recovery period) resulted in dose-related effects in male rats of diminished body weight gains, decreased organ weights, mortality, decreased reproductive performance and fetal viability, decreased sperm number in the testes and epididymis, and altered sperm morphology (Linder et al., 1982; JRDI14). Based on the findings of testicular toxicity it is recommended that a special study of at least three months' duration be performed to establish a NOEL for testicular effects.

H. Structure-Activity Relationships (SAR)

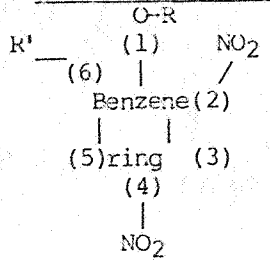
Dinoseb is structurally similar to dinitrophenol (DNP) and a number of other DNP analogs which have a similar metabolic action of stimulation of oxygen consumption through an inhibition of oxidative phosphorylation (Hayes, 1982; JRDI26). A summary table of toxicological endpoints is presented below.

Dinoseb along with DNP and dinitro-o-cresol(DNOC) are quite toxic being in Category I or II for acute oral toxicity. Due to their larger side chains, which reduce their rate of absorption, 2-sec-butyl-4,6-dinitrophenyl-3-methylcrotonate (binapacryl) and dinitrooctyl phenol(dinocap) have lower acute toxicities, although it should be noted that the intravenous $LD_{50}$ in male rats for Dinocap is 2.3 mg/kg as compared to a $LD_{50}$ of 40 mg/kg for Dinoseb in rats (oral intubation).

Generally speaking, the DNP analogs are reported as cataractogenic in humans and a number of animal species, including ducklings (a consistent observation), chickens, dogs, mice and rabbits. Interestingly, rats appear to be refractory to the cataractogenic effects of the dinitrophenols.

Another consistent findings is the observation of reproductive/testicular effects for Dinoseb, dinitrophenol and other analogs. Although binapacryl did not show any reproductive toxicity the dosage tested was relatively low. The SAR data also suggests that dinitrophenol analogs may be teratogenic, although test results have only been reported for two analogs, Dinocap and Dinoseb, at the present time.

Findings related to oncogenicity and mutagenicity are inconclusive. Additional testing is required to clarify these issues.

| Structural Analog* | Acute Toxicity | Produces Cataracts? | Oncogenic/ Mutagenic | Reproductive/ Testicular | Teratogenic |
|---|---|---|---|---|---|
|  | | | | | |
| Dinitrophenol (2,4-dinitro-; R & R'= H) | Category I-II | Yes:humans, ducklings, chickens | Under test in NTP(FY 85) for muta | Testicular atrophy in rats | Not tested |
| 2,4-Dinitrocresol (DNOC) (R=H; R'= $CH_3$) | Category I | Yes:humans, ducklings | Chromosoral aber. pos. in vitro, in vivo | Embryos increased chromsomal aber.; damaged sperm in mice | Not tested |
| 2-sec-butyl-4,6-dinitrophenyl-3-methylcrotonate (Dinapacryl) (R=-C(=O)-CH=C($CH_3$)-; R'=-CH($C_2H_5$)-$CH_3$) | Category II-III | No:rats, dogs | Not tested | No effect in 3-gen repro at 50 ppm(HDT) in rats | Not tested |
| Dinitrooctyl phenol (Dinocap) (R=-C-CH=CH-$CH_3$; R'=-$(CH_2)_5$-$CH_3$) | Category II-III | Yes:ducklings†, rabbits†, (dogs†¶) | Incon for onco†/pos. & neg. for mutag† | Decreased conception: male or female effect†(rats) | Incon:NTD not tested, fetotoxic† in mice, rabbits, +/- in rats |
| 2-sec-butyl-4,6-dinitro phenol (Dinoseb¶) (R= H; 2 position= sec-butyl) | Category I | Yes:humans, ducklings, dogs,mice | Incon for onco in mice/pos.† & neg. for mutag† | Yes:decreased pup wts/decreased sperm ct & morphology | Yes in mice by ip & oral |

* information taken from Hayes, 1982: DRU126 or EPA reviews : 1) cataracts: review of 2 ocular toxicity studies with Karathane; Q. Bui to J. Ellenberger; 5/2/85, 2) oncogenicity: one liners, 3) mutagencity: one liners, 4) reproductive/testicular: same review discussed above for 1), 5)teratogenicity: one liners †retinal atrophy reported in two year dog study: one liners; ¶data from Registration Standard

# APPENDIX I: HARRISON TABLES

TABLE A
GENERIC DATA REQUIREMENTS FOR DINOSEB

| Data Requirement | Composition 1/ | Use Patterns 2/ | Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially) | Bibliographic Citation | Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)? 3/ |
|---|---|---|---|---|---|
| §158.135 Toxicology | | | | | |
| ACUTE TESTING: | | | | | |
| 81-1 - Acute Oral - Rat | TGAI | A,B | Yes | 90370;67698;60749 | No |
| 81-2 - Acute Dermal | TGAI | A,B | No | | Yes |
| 81-3 - Acute Inhalation - Rat | TGAI | A,B | No | | Yes |
| 81-4 - Eye Irritation - Rabbit | TGAI | A,B | No | | Yes |
| 81-5 - Dermal Irritation - Rabbit | TGAI | A,B | No | | Yes |
| 81-6 - Dermal Sensitization - Guinea Pig | TGAI | A,B | No | | Yes 4/ |
| 81-7 - Acute Delayed Neurotoxicity - Hen | TGAI | A,B | N/A | | N/A |
| SUBCHRONIC TESTING: | | | | | |
| 82-1 - 90-Day Feeding - Rodent | TGAI | A,B | No | | No 5/ |
| Non-rodent | TGAI | A,B | No | | No 5/ |

TABLE A
GENERIC DATA REQUIREMENTS FOR DINOSEB

| Data Requirement | Composition 1/ | Use Pattern 2/ | Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)? | Bibliographic Citation | Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)? 3/ |
|---|---|---|---|---|---|
| §158.135 Toxicology (Cont.) | | | | | |
| 82-2 - 21-Day Dermal- | TGAI | A,B | No | JRDI22 | Yes |
| 82-3 - 90-Day Dermal- | TGAI | A,B | N/A | | N/A |
| 82-4 - 90-Day Inhalation - Rat | TGAI | A,B | N/A | | N/A |
| 82-5 - 90-Day Neurotoxicity- | TGAI | A,B | N/A | | N/A |
| CHRONIC TESTING: | | | | | |
| 83-1 - Chronic Toxicity - | | | | | |
| Rodent | TGAI | A,B | No | | Yes 6/ |
| Non-rodent | TGAI | A,B | No | | Yes |
| 83-2 - Oncogenicity Study - | | | | | |
| Rat | TGAI | A,B | No | | Yes |
| Mouse | TGAI | A,B | No | 25582 | No 7/ |
| 83-3 - Teratogenicity - | | | | | |
| Rat | TGAI | A,B | No | | Yes |
| Rabbit | TGAI | A,B | No | | Yes |
| 83-4 - Reproduction, 2-generation | TGAI | A,B | No | | Yes 8/ |

TABLE A
GENERIC DATA REQUIREMENTS FOR DINOSEB

| Data Requirement | Composition 1/ | Use Pattern 2/ | Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially) | Bibliographic Citation | Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)? 3/ |
|---|---|---|---|---|---|
| §158.135 Toxicology (continued) | | | | | |
| MUTAGENICITY TESTING | | | | | |
| 84-2 - Gene Mutation | TGAI | A,B | Partially | 132582,5009139,24901; JRDI08;JRDI16 | Yes 9/ |
| 84-2 - Chromosomal Aberration | TGAI | A,B | No | | Yes |
| 84-2 - Other Mechanisms of Mutagenicity | TGAI | A,B | Yes | 132582,5009139,24901; 24901 | No |
| SPECIAL TESTING | | | | | |
| 85-1 - General Metabolism | PAI or PAIRA | A,B | No | | Yes |
| 85-2 - Domestic Animal Safety | Choice | A,B | N/A | | N/A |

1/ Composition: PAI = Pure active ingredient; PAIRA = Pure active ingredient, radiolabelled; Choice = Choice of several test substances determined on a case-by-case basis.
2/ The use patterns are coded as follows: A=Terrestrial, Food Crop; B=Terrestrial, Non-Food; C=Aquatic, Food Crop; D=Aquatic, Non-Food; E=Greenhouse, Food Crop; F=Greenhouse, Non-Food; G=Forestry; H=Domestic Outdoor; I=Indoor.
3/ Data must be submitted no later than six months after the publication of this Standard except for the following tests: 82-2/7 months; 83-1/42 months; 83-2/42 months; 83-3/12 months; 83-4/20 months; 84-2/10 months; 85-1/14 months.
4/ There is no evidence, based on Dinoseb's chemical structure, to suggest that Dinoseb is a neurotoxic compound.
5/ Subchronic oral studies are unnecessary since chronic studies are required to support permanent tolerances.
6/ There are findings of an effect of Dinoseb in the hamster of interference with the development of normal delayed type hypersensitivity response and possible suppression of circulating antibody, as well as the observation in a chronic mouse study of an apparent treatment-related increase over control incidence of thymus atrophy. Therefore, special emphasis on immune-related tissues (spleen, thymus, liver, bone marrow, blood) in the histopathological examination is required. Periodic measurement of circulating serum immunoglobulins during the study should also be considered.

6/ (continued)
In addition, due to the observation of lenticular opacities in several species(mouse, duckling, rabbits and dogs), establishment of the dose-related effects of dinocap on lenticular opacities(cataracts) is required.

7/ It is highly probable that the mouse study will be upgraded to Core Minimum and thus fulfill a requirement for one species, the mouse.

8/ Based on the findings of significant testicular toxicity in the rat, including decreased sperm number in the testes and epididymis, and altered sperm morphology--which did not return to normal values after a lengthy (16-week) recovery period, a special test to establish a NOEL for testicular effects must be performed. This test may be incorporated into the protocol for the reproductive test, and approval of the protocol by to the Agency prior to initiation of the study.

9/ Acceptable studies in bacterial assays were negative but no studies in mammalian cell assays were performed.

TABLE B

41 PRODUCT SPECIFIC DATA REQUIREMENTS FOR MANUFACTURING-USE PRODUCTS CONTAINING DINOSEB

| Data Requirement | Composition 1/ | Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially) | Bibliographic Citation | Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)? 2/ |
|---|---|---|---|---|
| §158.145 Toxicology | | | | |
| ACUTE TESTING | | | | |
| 81-1 - Acute Oral - Rat | MP | Yes | 90370;67698;60749 | No |
| 81-2-Acute Dermal | MP | No | | Yes |
| 81-3 - Acute Inhalation-Rat | MP | No | | Yes |
| 81-4-Primary Eye Irritation-Rabbit | MP | No | | Yes |
| 81-5-Primary Dermal Irritation | MP | No | | Yes |
| 81-6-Dermal Sensitization | MP | No | | Yes |

1/ Composition: MP= Manufacturing-use product
2/ Data must be submitted no later than 6 months after publication of this Standard.

APPPENDIX II: BIBLIOGRAPHY

# BIBLIOGRAPHY

MRID/ID #


00024901
00132582
05009139 Simmon V.F., Mitchell A.D., Jorgensen T.A. (1977) Evaluation of selected pesticides as chemical mutagens: in vitro and in vivo studies. Stanford Research Laboratory; Report No. EPA-600/1-77-028, contract no. 68-01-2458 (Technical Report/Environmental Health Effects Series Report) dated 1977.

00025582 Piccirillo, V.J., Banas, D.A. (1977) 104-Week dietary study in rats: Dinoseb DNBP. Final Rept. (unpublished study including letter dated Mar 2, 1979 from F.E. Reno to Art E. Schober, received Dec 13, 1979 under 464-490; prepared by Hazleton Laboratories America, Inc.; submitted by Dow Chemical U.S.A., Midland, Mich.; CDL:241509-B).

00025579 Jensen, D.J. (1978) Monitoring of dinoseb formulations for N-nitrosdiethanol amine. (Unpublished study received Dec 13, 1979 under 464-490; submitted by Dow Chemical U.S.A., Midland, Mich.; CDL: 241509-B).

00033636
00060766 Ernst, W., Baer, F. (1964) The conversion of 2,4-dinitro-6-sec-butylphenol and its esters in the animal organism. A translated abstract of: Die Umwandlung Des 2,4-dinitro-6-sec.-Butylphenols Forschung 14(2): 81-84. (translated from: Chemical Abstracts 60: 16362F: unpublished study, including German text, received Jun 10, 1968 under 8F0712.

00039368
00057711 Gibson, J.E. (1973) Teratology studies in mice with 2-sec-butyl-4,6-dinitrophenol (Dinoseb). Food and Cosmetic Toxicology 11(?): 31-45. (Also/in/unpublished submission received Nov 11, 1970 and Feb 14, 1973 under 1F1075; submitted by Dow Chemical U.S.A., Midland, Michigan; CDL:092140-G, CDL:090831-H).

00039369 Gibson, J.E., Rao, K.S. (1973) Disposition of 2-sec-butyl-4,6-dinitrophenol (Dinoseb) in pregnant mice. Food and Cosmetics Toxicology 11(?): 45-52. (also/in/unpublished submission received Feb 14, 1973 under 1F1075: submitted by Dow Chemical U.S.A., Midland, Mich.; CDL:092140-E).

00060767 Henneberg, M. (1964) Metabolites of dinitroisopropylphenol (DNPP) and of dinitrobutylphenol (DNBP) in the rat organism. Pages 233-237,/in/ without title. By ? M.P. A translation of: metabolity dwunitroizopropyl ofenolu (DNPP) I dwunitrobutylofenolu (DNBP) W organizmie szczurow. Acta Poloniae Pharmaceutica XXI(2):222-223. (also/in/unpublished submission, including German text, recd Jun 10, 1968 under 8F0712; submtd by Dow Chem. USA, Midland, Mich.:CDL:091234-J).

00060749 Wazeter, F.X., Long, J.E. (1968) Acute toxicity in mice: IRDC No. 133-030. (Unpublished study received Jun 10, 1968 under 8F0712; prepared by International Research and Development Corp., submitted by Dow Chemical U.S.A., Midland, Mich.; CDL:091234-A).

00067698 Rowe, V.K., McCollister, D.D., Spencer, H.C. (1949?) The acute oral toxicity of 2-sec-butyl-4,6-dinitrophenol, triethanolamine salt to rats, guinea pigs and chicks. (unpublished study received on unknown date under 7F0593; submitted by Dow Chemical Co., Indianapolis, Ind.; CDL:092881-E).

00067701 Gay, H.H. (1951) Results of ocular examination of persons employed
00090369 in the manufacture and handling of dinitrophenols: (1) Dinitro-o-sec. Butylphenol; (2) Dinitro-o-sec-cyclohexylphenol. (unpublished study received Dec 23, 1959 under 7F0593; submitted by Dow Chemical Co., Indianapolis, Ind.; CDL:092881-K).

00070514 Kaminiski, F., Rothman, L.D., Kracht, W.R. (1978) Analyses for N-nitrosodiethanolamine in Premerge-(TM)° and Premerge 3 Herbicides: ML-AL 78-00368. (Unpublished study received Feb 25, 1981 under 464-490; submittted by Dow Chemical U.S.A., Midland, Mich.; CDL: 244483-A).

00090300 Lynn, G.E. (1966) Letter sent to Justus C. Ward dated Feb 11,1966 concerning basal metabolism rates of workmen exposed to 4,6-dinitro-o-sec.butylphenol. (unpublished study, including letter dated from Oct 1, 1965 from G.E. Lynn to Justus C. Ward and undated letter from J.C. Ward to G.E. Lynn, received on unknown date under unknown admin. no.: submitted by Dow Chemical U.S.A., Midland, Mich.; CDL:130434-A).

00090368 Lynn, G.E. (1965) Letter sent to Justus C. Ward dated Oct 1, 1965 concerning toxicity of 2,4-dinitro-o-sec-butylphenol in humans. (unpublished study received on unknown data under unknown admin. no.; submitted by Dow Chemical U.S.A., Midland, Mich.,; CDL:130443-A).

00090370 Rowe, V.K., McCollister, D.D., Spencer, H.C. (1949?) The acute oral toxicity of 2-sec.butyl-4,6-dinitrophenol to rats, guinea pigs, and chicks. (unpublished study received on May 6,1965 under unknown admin. no.; submitted by Dow Chemical U.S.A., Midland, Mich.,CDL: 130443-D).

00090374 Spencer, H.C., Rowe, V.K., Adams, E.M. et al. (1948) Toxicological studies on laboratory animals of certain alkyldinitrophenols used in agriculture. J. Industrial Hygiene and Toxicology 30(1):10-25. (also/in/unpublished submission received May 6, 1965 under unknown admin. no.; submitted by Dow Chemical U.S.A., Midland, Mich.; CDL:130443-T).

00114252 Dandliker, W, Hicks, A., Levison, S. et al. (1980) Effects of pesticides on the immune response. Environmental Science and Technology 14(2):204-210. (submitted 3681; also in unpublished submission received Sep 7,1982 under 1258-517; submitted by Clin Corp., Stanford, CT; CDL:248286-X).

00114962 McCollister, D., Copeland, J., Oyen, F. (1967) Results of 91-day dietary feeding studies of 2-secondary butyl-4,6-dinitrophenol (DNOSBP) in beagle hounds. (unpublished study received April 13, 1967 under 7F0593; submitted by Dow Chemical Co., Indianapolis, In; CDL:080764-C).

00114963 Tucker, W., Bennett, B., Robinson, V. (1967) Oral toxicity study of 2-sec-butyl-4,6-dinitrophenol in White Pekin ducklings. (unpublished study received April 13, 1967 under 7F0593; submitted by Dow Chemical Co., Indianapolis, In; CDL:090764-D).

00114965 Dow Chemical Co. (1956) Basal metabolism rates and exposure data on workmen employed in the manufacture of ... DNOBSP. (unpublished study received April 13, 1967 under 7F0593; CDL:090764-F).

00500588 Gilberg, B.O. (1971) On the effects of some pesticides on rhizobium and isolation of pesticide-resistant mutants. Archives of Microbiology 75(3): 203-208.

05001460 Anderson, V.F., Leighty, E.G., Takahashi, M.I. (1972) Evaluation of herbicides for possible mutagenic properties. Journal of Agricultural and Food Chemistry 20(3); 649-656.

05016148 Toure, I.M., Stenz, E. (1977) Der einfluss ausgewaehuter herbizide auf bakteriophagen und Escherichia coli. The action of selected herbicides on bacteriophages and Escherichia coli. Zentralblatt fuer Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abteilung TI 132:163-177.

JRDI02 Beaudoin, A.R., Fischer, D. (1981) An in vitro evaluation of teratogenic action. Teratology, 23:57-61.

JRDI03 Brown, D. (1981). Dinoseb: a 100 week oral (dietary) toxicity and carcinogenicity study in the mouse. Performed at Hazleton Laboratories Europe Ltd., Otley Rd., Harrogate, HG3 1PY, England. Study no. 1839-50/20; June 1981; Acc. No. 259494-259498; EPA # 54299-Q(1).

JRDI04 Chernoff, N., Kavlock, R.J. (1982) An in vivo teratology screen utilizing pregnant mice. J. Tox. Envir. Health, 10: 541-550.

JRDI06 Edgerton, T.R., Moseman, R.F. (1978) Electron-capture gas chromatographic determination of 2-sec-butyl-4,6-dinitrophenol (DNBP) residues in feed, tissue, and excreta. J. Agr. Food Chem., 26(2):425-428.

JRDI07 Edson, E.F. (1969) Chemical hazards in agriculture. Ann. Occupational Hyg., 12(2): 99-108.

JRDI08 Eisenbeis, S.J., Lynch, D.L., Hampel, A.E. (1981) Ames mutagen assay tested against herbicides and herbicide combinations. Soil Sci., 131: 44-47.

JRDI09 Gray, L.E. Jr., Kavlock, R.J. (1984) An extended evaluation of an in vitro teratology screen utilizing postnatal growth and viability in the mouse. Teratog., Carcinog., Mutagen., 4(5): 403-426.

RDI11 Irvine,L.F.H. (1981). Dinoseb three generation reproductive performance study in the rat (dietary) Hazleton Europe(#2006-50/19). Performed at Hazleton Laboratories Europe Ltd., Otley Rd., Harrogate, HG3 1PY, England. Study no. 1839-50/20; June 1981; Acc. No. 259499-259506; EPA # 54299-Q(2); August 1981.

Irvine,L.F.H. (1981). 2-sec-butyl-4,6-dinitrophenol(dinoseb) additional 2 generation phase of a 3 generation reproductive performance study in the rat (dietary). Performed at Hazleton Laboratories Europe Ltd., Otley Rd., Harrogate, HG3 1PY, England. Study no. 2350-50/58; Acc. No. 259499-259506; EPA # 54299-Q(2); April 1981.

RDI13 Kavlock, R.J., Chernoff, N., Rogers, E.H. (1985) The effect of acute maternal toxicity on fetal development in the mouse. Teratogen., Carcinog., Mutagen., 5(1): 3-14.

RDI14 Linder, R.E., Scotti, T.M., Svendsgaard, D.J. et al. (1982) Testicular effects of dinoseb in rats. Arch. Environ. Contam. Toxicol., 11(4): 475-485.

RDI15 McCormack, K.M., Abuelgasim, A., Sanger, V.L., Hook, J.B. (1980) Postnatal morphology and functional capacity of the kidney following prenatal treatment with dinoseb in rats. J. Toxicol. Environ. Health, 6(5): 633-644.

RDI16 Moriya, M., Ohta, T., Watanabe, K. et al. (1983) Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mutat. Res., 116:185-216.

RDI17 Osterloh, J., Letz, G., Pond, S., Becker, C. (1983) An assessment of the potential testicular-toxicity of 10 pesticides using the mouse-sperm morphology assay. Mutat. Res., 116:407-415.

RDI18 Preache, M.M., Gibson, J.E. (1975) Effects in mice at high and low environmental temperature on the maternal and fetal toxicity of 2-sec-butyl-4,6-dinitrophenol (Dinoseb) and on disposition of $^{14}C$-dinoseb. Teratology, 12:147-156.

RDI19 Preache, M.M., Gibson, J.E. (1975) Effect of food deprivation, phenobarbital and SKF-525A on teratogenicity induced by 2-sec-butyl-4,6-dinitrophenol (dinoseb) and on disposition of ($^{14}C$)dinoseb in mice. J. Toxicol. Environ. Health, 1:107-118.

RDI20 Shirasu, Y., Moriya, M., Tezuka, H. et al. (1982) Knowledge gained from the testing of large numbers of chemicals in a multi-laboratory, multi-system mutagenicity testing program. Environ. Mutagens Carcing. Proc. Int. Conf., 3rd(1981): 331-335.

RDI21 Siebert, D., Lemperle, E. (1974) Genetic effects of herbicides: Induction of mitotic gene conversion in Saccharomyces cerevisiae. Mutat. Res., 22(2): 111-120.

DI22 Smith, W.D.L. (1981) An investigation of suspected dinoseb poisoning after the agricultural use of a herbicide. Practitioner, 225(1356): 923-926.

DI23 Spencer, F., Sing L.T. (1982) Reproductive toxicity in pseudopregnant and pregnant rats following postimplantation exposure: Effects of the herbicide dinoseb. Pestic. Biochem. Physiol., 18(2): 150-57.

DI24 Waters, M.D., Simmon, V.F., Mitchell, A.D. et al. (1980) Overview of short-term tests for the mutagenic and carcinogenic potential of pesticides. J. Environ. Sci. Health, Part B, 15: 867-906.

DI25 Waters, M.D., Sandhu, S.S., Simmon, V.F. et al. (1982) Study of pesticide genotoxicity. Basic Life Sci., 21: 275-326.

DI26 Hayes,Jr., W.J. (1982) Chapter 9. Nitro compounds and related phenolic pesticides In: Pesticides studied in Man. Williams & Wilkins, Baltimore/London.

APPENDIX III: ONE LINERS

ONE-LINER INDEX

# APPENDIX IV: DATA EVALUATION REPORTS

005421

STUDY EVALUATION

Secondary Reviewer

| | |
|---|---|
| Study Title: | The acute oral toxicity of 2-sec-butyl-4,6-dinitrophenol to rats, guinea pigs and chicks |
| Reference: | MRID 90370 |
| Testing Facility: | Biochemical Research Department, The Dow Chemical Company, Midland, Michigan |
| Final Report No.: | Not stated |
| Final Report Date: | Not stated |
| Study Authors: | Rowe V.K., et al. |
| Sponsor: | Dow Chemical Company |
| Test Material: | 2-sec-Butyl-4,6-dinitrophenol dissolved in olive oil and emulsified with 5-10 percent gum arabic |
| Dose Levels: | 0.005 to 0.060 gm/kg |
| Species: | Rats(white, mature), guinea pigs(mature) from stock colonies from the laboratory; chicks, strain New Hampshire Red, aproximately 3 weeks of age |

METHODS:

Single oral doses of 2-sec-butyl-4,6-dinitrophenol (DNBP) were administered by intubation to mixed sex groups of rats (10 to 20/dose), guinea pigs (5/dose), and chicks (2 to 5/dose). The volume of oil administered to any species was usually 1 ml but not more than 4 ml. Mortalities of animals were recorded; animals were observed up to two weeks. The acute oral $LD_{50}$ values were determined with their 95% confidence limits by the method of Litchfield and Wilcoxon (1949) along with the slope function.

Comments:

1. Test substance not adequately identified.

2. Number of animal/sex/dose not completely identified.

3. Unclear whether or not the animals received the same volume of dosing solution.

4. No clinical, individual animal weight or necropsy data submitted.

RESULTS/CONCLUSIONS/RECOMMENDATIONS:

The following acute oral $LD_{50}$ values were determined:

| | $LD_{50}$ (95% C.L.) | slope function† |
|---|---|---|
| rats | 0.040(0.032-0.050)* | 2.00 |
| guinea pigs | 0.025(0.020-0.031) | 1.42 |
| chicks | 0.026(0.018-0.037) | 1.77 |

† stated as the fold change in dosage required to produce one unit standard deviation in response along the line
* significantly different ($p<0.05$) from guinea pigs and chicks

The Litchfield and Wilcoxon test for parallelism of two lines and estimate of relative potency indicated that DNBP was more toxic to guinea pigs and chicks than to rats. The guinea pigs and chicks had similar acute toxicities and dose-response slopes.

This study is designated as Core Supplementary data based on the comments for the methods.

005121

STUDY EVALUATION

Secondary Reviewer

| | |
|---|---|
| Study Title: | Acute toxicity studies in mice |
| Reference: | MRID 60749 |
| Testing Facility: | International Research and Development Corp. |
| Final Report No.: | IRDC No. 133-030 |
| Final Report Date: | February 8, 1963 |
| Study Authors: | Wazeter, F.X. and Long, J.E. |
| Sponsor: | Dow Chemical Company |
| Test Material: | DNBP(AGR. No. 16053-B0; 2-sec-butyl-4,6-dinitrophenol and analogs); only DNBP test reviewed |
| Dose Levels: | Dosage levels ranging from 14.7 to 68.1 mg/kg |
| Species: | Mice, albino males; Charles River strain |

METHODS:

Mice of 18 to 24 grams weight were housed five per cage with food and water available ad libitum except during a six-hour fasting period prior to compound administration during which food, but not water, was withdrawn. The test compound was diluted with corn oil (Mazola) and administered orally. The volume was maintained constant at one ml per 100 grams of body weight.

The mice were observed for phamacodynamic and/or toxic signs and mortality continuously for five hours immediately following compound administration, and at least once daily thereafter for a period of ten days.

Comments:

1. Test substance not adequately identified.

2. Only males were used; females could be more sensitive.

3. Animals not observed for 14 days after dosing.

4. No individual clinical, animal body weight or necropsy data provided.

RESULTS/CONCLUSIONS/RECOMMENDATIONS:

Oral dosing resulted in the following response (deaths): 14.7 mg/kg=1/5; 21.5 mg/kg=0/5; 31.6 mg/kg=0/5; 46.4 mg/kg=4/5; 68.1 mg/kg=5/5. The $LD_{50}$ was calculated as 41.4 with 95% confidence limits of 35.5-48.2 mg/kg.

A frequent clinical sign was salivation of the mice soon after dosage administration (within five hours) and at the three highest doses, reduction in general activity. One high dose mouse showed reduced cardiac and respiratory rates.

This study is designated as Core Supplementary data.

STUDY EVALUATION

Secondary Reviewer

| | |
|---|---|
| Study Title: | The acute oral toxicity of 2-sec-butyl-4,6-dinitrophenol, triethanolamine salt to rats, guinea pigs and chicks |
| Reference: | MRID 67698 |
| Testing Facility: | Biochemical Research Laboratory, Dow Chemical Company, Midland, Michigan |
| Final Report No.: | Unknown |
| Final Report Date: | Unknown |
| Study Authors: | Rowe V.K., et al. |
| Sponsor: | Dow Chemical Company |
| Test Material: | 2-sec-Butyl-4,6-dinitrophenol triethanolamine salt as 1 per cent aqueous solution |
| Dose Levels: | 0.04 to 0.25 gm/kg |
| Species: | Rat(albino, males) and guinea pigs(mixed sexes) from stock colonies of the laboratory; chicks, three weeks old, mixed sexes (New Hampshire Red strain) |

METHODS:

Single oral doses of test compound were administered by intubation. The volume of solution given did not exceed 7 ml for the rats or guinea pigs or no more than 2 ml for the chicks. All surviving animals were observed until they were fully recovered (usually 14 days). The $LD_{50}$, 95% confidence limits and the slope function were determined for each species by the method of Litchfield and Wilcoxon (1949).

Comments:

1. The test substance was not adequately identified.

2. Number of animals/sex/dose not identified for the guinea pigs or chicks.

3. Unclear whether or not the animals received the same volume of dosing solution. The dosing volume may have been too high but this cannot be determined without body weight data.

4. No clinical, individual animal weight or necropsy data submitted.

RESULTS/CONCLUSIONS/RECOMMENDATIONS:

Results of the study are presented below:

| | $LD_{50}$ (95% C.L.)gm/kg | slope function† |
|---|---|---|
| rats (male) | 0.114(0.089-0.146) | 1.32 |
| guinea pigs | 0.088(0.080-0.097) | 1.11 |
| chicks | 0.070(0.048-0.103)* | 1.41 |

† stated as the fold change in dosage required to produce one unit standard deviation in response along the line
* significantly different ($p<0.05$) from rats

There was a significant difference in the $LD_{50}$ value between the rats and chicks or guinea pigs, the rats being less sensitive to the acute toxicity of the DNBP triethanolamine salt than the other two species.

This study is designated as Core Supplementary data.

005421

STUDY EVALUATION

W. Porter
Secondary Reviewer

| | |
|---|---|
| Study Title: | Toxicological studies on laboratory animals of certain alkyldinitrophenols used in agriculture |
| Reference: | MRID 90374 |
| Testing Facility: | Biochemical Research Laboratory, The Dow Chemical Company, Midland, Michigan |
| Final Report No.: | J. Industr. Hyg. Toxicol. 30(1):10-25; CDL 130443-I |
| Final Report Date: | January, 1948 |
| Study Authors: | Spencer, H.C. et al. |
| Sponsor: | Dow Chemical Company, Midland, Michigan |
| Test Material: | Several alkyldinitrophenols including dinoseb(2-sec-butyl 4,6-dinitrophenol) with a stated purity of 99.1 to 99.7%; dark amber crystals (monoclinic form) |
| Dose Levels: | Dermal studies: 3% alcoholic solution or 10% solution in butylcarbitol acetate for rabbits; several ethanolic solutions (equivalent to 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.6, and 1.0 g/kg) in guinea pigs<br>Single oral doses: 0.005, 0.010, 0.020, 0.023, 0.027, 0.030, 0.040, 0.050, 0.060 g/kg) of dinoseb dissolved in olive oil and emulsified with 5-10% gum arabic solution<br>Six month feeding: 0.00, 0.005, 0.01, 0.020, 0.05 %(weight %) of diet in rats<br>Feeding study(ducks): 0.25% for 3 days, 0.10% for 4 days, 0.03% for 8 days (weight %) in diet |
| Species: | Dermal studies: rabbits, white (sex not specified)/3 per dose level; guinea pigs (both sexes), 5 per dose level<br>Single dose oral studies: rats, white male from Breeding and Laboratory Institute, Brooklyn, NY, 10 to 20 per dose level<br>Six month feeding: same as acute study, 30 per dose and 10 to 20 per dose level<br>Feeding study in ducks: ducklings, white Pekin from commercial hatchery, 8 to 10 per dose level |

METHODS:

Skin irritation and absorption

Rabbits: The method of Adams et al., 1941 (Industr. Med., 10: Ind. Hyg. Sec. 2: 1-4) was employed with materials all tested as 3% solutions in 95% ethanol or (for dinoseb) with 10% solution in butylcarbitol acetate. Routinely, 20 applications were made to the ear and 20 applications were bandaged onto the shaven abdomen of each rabbit over a period of four weeks.

Guinea pigs: A single dose in alcoholic solution was applied to the clipped abdomen of each animal (5 animals/dose). Each animal was restrained in such a manner that the treated area could be kept wet with ethanol during the four-hour period following the application of the test material in order to facilitate its absorption. At the end of this period, the surviving animals were removed from the boards, bandaged so as to prevent oral ingestion, caged and observed until it was certain that they were fully recovered.

Rats/single oral dose: The volume of oil given to each rat was always less than 3 ml and usually of the order of 1 ml. All of the rats that survived were observed until recovery was complete (usually about 2 weeks).

Rat/dietary study for six months: A modified Sherman diet was used as the stock ration for the animals. The experimental diets were prepared by thoroughly mixing the dinoseb, which was in a flour concentrate, with the stock diet on a per cent by weight basis using a mechanical mixer and the concentration of the test material in the test diet was checked by chemical analysis. Each flour concentrate was prepared by adding wheat flour to an alcohol solution of the test material to form a thick paste which was then dried, ground, passed through an 80-mesh sieve, and analyzed. The use of these concentrates facilitated the accurate addition of small quantities of the test materials to the basic diet. The diets were made up from freshly prepared stock diet as needed. No diet preparations over a month old were used during the course of the study. The rats were fed from stainless-steel hoppers which were weighed and refilled three times a week.

Rats from the Breeding and Laboratory Institute were received when about twenty-five to thirty days old (although the exact ages were not known), maintained for three to four weeks on the stock diet, and then divided according to body weights into matched groups and started on the experimental diets. Five rats were caged together in wire bottom cages. The animals had free access to food and water at all times. In addition, each rat was given approximately 3 grams of cabbage twice weekly. The rats were weighed twice a week and records were kept of the body weight, general appearance, and estimated average food consumption of each animal. Animals that died were examined for gross pathological lesions.

Periodic hematological examinations were made on several groups of animals for erythrocyte count, hemoglobin concentration, total leucocyte count and differential count.

At the end of the study all of the surviving rats were starved overnight, weighed, killed by decapitation, and examined. The liver, kidneys, heart, and testes were weighed; and the following tissues from representative animals in each group were saved for histopathological studies: lung, heart, liver, kidney,

59

spleen, adrenal, pancreas, testis, stomach, and bone marrow (H-E sections). The concentration of urea nitrogen in the blood was determined at necropsy. Bone marrow counts were made on many of the rats which were examined for hematology parameters. The t-test was used for statistical analysis of control versus experimental groups.

Feeding study in ducks: Five-day old Pekin ducklings were purchased from a commercial hatchery, maintained on Purina Duck Startena for about a week, and then started on diets prepared by thoroughly mixing definite quantities of dinoseb with the Startena. The 8 to 10 ducklings in each group were examined frequently for body weight changes, observable ill effects, and particularly cataract formation. The ducklings were fed up to eight days with the experimental diet.

Comments:

This study in 1948 was not designed to meet the present regulatory requirements for any of the particular types of studies for which it might be considered appropriate including acute oral, acute dermal, repeated dose dermal (21 day) or subchronic studies. There are numerous deficiencies for each type of study according to current guidelines.

RESULTS:

Dermal application of dinoseb (3% ethanolic solution; volume not stated) to the rabbit ear resulted in no apparent irritation; however when applied onto the shaven abdomen of 3 different rabbits, death occurred after 1, 3 and 8 exposures, respectively. No irritation of the abdominal skin was observed. A 10% solution of dinoseb(volume not stated) in butylcarbitol acetate applied (abdomen and ear) to 3 rabbits resulted in death for all animals, again without any evidence of dermal irritation.

Dermal application of dinoseb (single dose in ethanol) resulted in dose-related mortality in guinea pigs with a "survival dose" (largest dose with all animals treated surviving) of 0.1 g/kg and a "lethal dose" (smallest dose causing death of all animals treated) of 0.5 g/kg. Dinoseb was found to be the most toxic of the dinitrophenols tested for dermal toxicity.

In acute oral toxicity tests (single dose) in rats the "survival dose" was found to be 0.005 g/kg and the "lethal dose" was 0.05 g/kg. Again dinoseb was the most acutely toxic of the compounds tested.

In the six month study in rats, the highest dose (0.05%) resulted in rapid loss of weight with four of the ten rats dying by day thirteen of treatment; the remaining six animals were sacrificed at 21 days and revealed marked emaciation with an average blood urea-N concentration of 55.0 mg per cent. Microscopic examination revealed slight degenerative changes in the renal tubules and slight cloudy swelling of the liver at the 0.05% dose level. Examination of the growth curves for dinoseb treated rats indicated that there was a small but consistent depression in body wt weights over the period of the study at the 0.02% dose level (3-8% below controls) which was apparently significant since comparison of the body weights for controls and treated animals at frequent intervals indicated p values which were slightly below 0.05 in most cases. There was a small but

statistically significant increase in liver weights (see Table 1 below) but other organ weights(kidney, heart or testes) were not affected by dinoseb administration at 0.02%. The average blood urea-N was 20.3 as compared with 17.5 mg% for the controls at the 0.02% dose level. No histopathological changes were reported. The growth curves for the lower dose groups (0.01 and 0.005%) were similar to the controls and no gross, clinical changes or histopathological changes were found.

Table 1: mean body weights[a] and organ weights[a]

| Dose (wt%) | #rats | body weight(g) | liver wt.(g) | kidney(g) | heart(g) | testes(g) |
|---|---|---|---|---|---|---|
| 0.00 | 21 | 278±4 | 7.13±.14 | 1.90±.05 | .95±.01 | 2.59±.06 |
| 0.005 | 18 | 286±5 | 6.83±.12 | 1.86±.04 | .95±.02 | 2.69±.07 |
| 0.01 | 16 | 281±5 | 7.47±.23 | 1.90±.07 | .96±.03 | 2.76±.10 |
| 0.02 | 17 | 266±6 | 7.85±.24* | 2.06±.07 | .96±.03 | 2.67±.08 |

[a] mean ± S.E.; *significantly different from control ($p<0.01$)

Table 2: % mortality and incidence of cataracts*in young ducklings

| # days on diet | controls (%) dead | controls (%) cataracts | 0.25% (%) dead | 0.25% (%) cataracts | 0.10% (%) dead | 0.10% (%) cataracts | 0.03% (%) dead | 0.03% (%) cataracts |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 56 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 56 | 0 | 63 | 0 | 20 | 0 |
| 3 | 0 | 0 | 100 | | 88 | 100 | 40 | 0 |
| 4 | 0 | 0 | | | 100 | | 50 | 0 |
| 5 | 0 | 0 | | | | | 50 | 20 |
| 6 | 0 | 0 | | | | | 50 | 20 |
| 7 | 0 | 0 | | | | | 50 | 20 |
| 8 | 0 | 0 | | | | | 50** | 40 |
| 18 | 0† | 0 | | | | | | |

* % living ducklings with cataracts; ** surviving ducklings accidently killed; † all surviving ducklings killed

Administration of dinoseb to Pekin ducklings in the feed (see Table 2 above) resulted in 100% mortality by day 3 of the diet at the high dose (0.25%) with no cataracts observed, 100% death by day 4 in the middle dose (0.10%) with one duckling having cataracts on day three of feeding, and 50% mortality by day 4 in the low dose (0.03%) with 40% of the animals with cataracts by day 8 (2 birds)--the first cataracts appearing on day 5. The authors noted that the rest of the low dose birds were accidentally killed on day 8.

DISCUSSION:

Dermal application of dinoseb to the rabbit (ear, abdomen) resulted in no apparent irritation but was quite acutely toxic by both routes of exposure. This indicates that substantial absorption through the dermis occurs from the ethanolic or butylcarbitol acetate solutions. Dermal application of dinoseb (single dose in ethanol) resulted in dose-related mortality in guinea pigs with a "survival dose" (largest dose with all animals treated surviving) of 0.1 g/kg and

a "lethal dose" (smallest dose causing death of all animals treated) of 0.5 g/kg. Dinoseb was found to be the most toxic of the dinitrophenols tested for dermal toxicity and the most acutely toxic of the compounds tested with a "lethal" oral dose of 0.05 g/kg.

Chronic dietary exposure to dinoseb for six months for rats did not result in the formation of cataracts at any of the doses tested (0.005, 0.01, 0.02, 0.05 wt. %). The highest dose (0.05%) resulted in rapid loss of weight with four of the ten rats dying by day thirteen of treatment; the remaining six animals were sacrificed at 21 days and revealed marked emaciation with an average Blood urea-N concentration of 55.0 mg per cent. Microscopic examination revealed slight degenerative changes in the renal tubules and slight cloudy swelling of the liver. Examination of the growth curves for dinoseb treated rats indicated that there was a small but consistent depression in body wt gains over the period of the study at the 0.02% dose level (3-8% below controls). There was a small but statistically significant increase in liver weights but other organ weights(kidney, heart or testes) were not effected by dinoseb administration at a 0.02% dose level. No discernible toxic effects were reported during the study or at necropsy for dose levels of 0.005 and 0.01%.

Pekin ducklings were very susceptable to the acutely lethal effects of dinoseb at doses of 0.25, 0.10 and 0.03% and the two lower doses resulted in some of the birds developing cataracts (100% cataracts at 0.1% by day 3, 40% cataracts at 0.03% by day 8). In comparison, 2,4-dinitrophenol (0.25%) produced 100% bilateral cataracts after only a 24 hour period on the experimental regimen.

This study conducted in 1948 was not designed to meet the present regulatory requirements for any of the particular types of studies for which it might be considered appropriate, including acute oral, acute dermal, repeated dose dermal (21 day) or subchronic studies. Based on numerous deficiencies when compared to current guidelines, this study is classified as Core Supplementary data.

005421

STUDY EVALUATION

Secondary Reviewer

Study Title: Oral toxicity study of 2-sec-butyl-4,6-dinitrophenol in White Pekin ducklings

Reference: MRID 114963

Testing Facility: Department of Pathology and Toxicology, Human Health Research and Development Laboratories, Dow Chemical Company, Zionsville, Indiana

Final Report No.: CDL: 090764-D

Final Report Date: January 23, 1967

Study Authors: Tucker, W.E.,Jr. and Bennett, B.K.

Sponsor: Dow Chemical Company

Test Material: Dinoseb, 2-sec-butyl-4,6-dinitrophenol, plant production lot no. 200136, 95.4 % purity

Dose Levels: Experiment I: dietary concentration of 0, 1, 3, 10, 30, 60, 100, 200, 300, 1000 ppm
Experiment II: 0, 100, 200, 300, 1000 ppm
Experiment III: 20, 40, 60, 80ppm

Species: Ducklings, White Pekin from a commercial flock; 10/dose group; sex not specified

METHODS:

Experiment I:

The ducklings were received when one day old and were five days old at the start of the test. They were housed in brooders with thermostatically controlled heating units for the first five days of the test and then transferred to 3'x 5' wire runs which had suspended wire bottom floors, automatic waterers and gravity feeders. Feed and water were available at all times except during one period of approximately twelve hours in which birds on the 200 ppm diet escaped from their pens overnight (on day 5).

All birds were killed after twenty-eight days except for four birds on the 60 ppm level and five controls. These were held for further observations and were killed after forty-eight days.

A standard commercial growth feed for ducks was used. For the control and treated diets the test material was dissolved in editle-grade corn oil prior to incorporation in the ration. The concentration of the compound in the solution was so that the addition of two parts (by wt.) of the solution to 98 parts of the feed resulted in the desired concentration of the compound in the finished diet. The basal diet was fed to the controls at all times and consisted of two parts solvent and 98 parts of the standard ration.

The birds were observed daily for general appearance. Certain treated groups were examined with a slit lamp and/or ophthalmoscope on days 1-5, 7-9, 26, 41, and 45 (only certain birds from the 60 ppm and control groups were examined on the on the 41st and 45th days). All birds in the control groups were examined on days 3 and 18. On other days only enough controls were examined to determine if changes similar to those seen in treated groups were present.

Average individual body weights (cage groups) were recorded weekly and records of food consumption of each group were kept and the approximate amount of chemical consumed by each group was calculated.

On the 28th day of the test, eyes from each of five birds from both the 300 ppm groups and control groups were removed and fixed in 10% formalin. After fixation the lenses were dissected from the eyes, processed and embedded in paraffin blocks. The imbedded lenses were then rough-cut from the anterior surface posteriorly until approximately the equatorial plane of the lens was reached. At that point a section was taken and stained with hematoxylin-eosin (H&E) for microscopic study. In addition, the lenses of birds from the 300 ppm level were further cut posteriorly until the tissue blocks were exhausted. Ten "step" sections were taken from the lens in the area between the equator and the posterior pole and stained with H&E. Eyes from several birds on the 60 ppm and control groups were taken after 48 days and handled in the same manner as above except that the lens was rough-cut from the side until approximately the median vertical plane of the lens was reached before a section was taken for microscopic study. This variation in sectioning was made in an attempt to demonstrate a peculiar peripheral vacuolar change the authors noted in both treated and control birds.

Experiment II:

This experiment was performed to check for possible early transient lesions at 100 and 200 ppm diets. Also, the 1000 ppm and 300 ppm levels were repeated to confirm observations made on these groups in the first study.

Methods were similar to those in experiment I except the duration of the study was for four days only. In addition the birds were examined daily by slit lamp for four consecutive days by the consulting ophthalmologist. Body weights, food and chemical consumption were not recorded. On day 2 the lenses from birds on the 1000 ppm level which showed lesions (central posterior subcapsular haziness and posterior sutural haziness) were processed and studied histologically. The lens were processed in the same manner as described in experiment I.

Experiment III:

This experiment was performed to determine a NOEL using lower dietary concentrations than those used in experiment II.

Duration was four days. Methods same as experiment I. No body weights, feed, or chemical consumption data were recorded. Histology was not done.

Comments:

This multi-experiment, ancillary study was designed to to answer a specific question regarding Dinoseb's cataractogenic properties in the duck. Inadequacies of the study include the following:

1. Inadequate identification of the test substance, its purity or stability.

2. A NOEL for lenticular opacities was not demonstrated in the long-term portion of the study.

3. Statistical analysis of the data was not performed.

RESULTS:

Dinoseb administration resulted in a dose-related depression in group average body weight gains (see Table 1 below) and death of all the ducks at the high dose level (1000 ppm) by day 5 of the study. It would appear that in the 100 ppm group there was an initial weight gain decrease for weeks 1 and 2 followed by recovery to within control values by day 21. The 200 ppm dosage level produced a consistent body weight gain reduction compared to control values. The decreased body weight gains are also reflected in the diminished food consumption observed at the 200 ppm dosage.

Table 1: Selected data for food consumption and body weight data (group averages)

| Food consumption(g) | Days of test 0 | 7 | 14 | 21 | 28 |
|---|---|---|---|---|---|
| control | --- | 57.4 | 127 | 171 | 217 |
| 60 ppm | --- | 50.7 | 129 | 192 | 213 |
| 100 ppm | --- | 45.8 | 162 | 204 | 239 |
| 200 ppm | --- | 37.9 | 98.6 | 143 | 183 |
| 300 ppm | --- | 32.8 | 59.1 | 120 | 164 |
| 1000 ppm | ----------all animals dead---------- | | | | |
| Body weight(g) | | | | | |
| control | 101.6 | 347 | 855 | 1360 | 1452 |
| 60 ppm | 92.0 | 310 | 790 | 1431 | 1482 |
| 100 ppm | 97.6 | 255 | 798 | 1424 | 1694 |
| 200 ppm | 96.2 | 220 | 553 | 949 | 1113 |
| 300 ppm | 94.1 | 130 | 273 | 580 | 748 |
| 1000 ppm | 92.6 | all dead---------- | | | |

Dose-related effects on the eye of the ducklings were reported (see Table 2 below) which were more apparent as the study progressed. This effects included central posterior subcapsular haziness of the lens, unilateral or bilateral sutural haziness, and unilateral or bilateral equatorial lenticular vacuolation (only observed at 60 ppm and in the controls). The author noted that several control birds were examined before treated birds through day 5 and central posterior subcapsular haziness of the lens was never seen. On days 3 and 18 all control birds were examined and all were reported negative.

As evident in Table 2, a condition stated as equatorial lenticular vacuolation (e.l.v.) was observed at day 26 in the 60 ppm group (1/10 ducks); at day 28, four ducks at the same dose were reported to have vacuolar changes and/or conjunctivitis. Two of four birds had e.l.v. at day 41 with none reported for controls. However by day 45, 2/4 60 ppm birds and 2/5 controls had this lens change.

In experiment II, performed to examine earlier, unexamined time periods for the 100 and 200 ppm doses, effects were observed as early as two days of treatment at 200 ppm [3/10 with posterior subcapsular haziness(p.s.h.)/sutural haziness (s.h.) or sutural haziness(s.h.)] and three days of treatment for 100 ppm(4/10 with s.h., or p.s.h. and s.h.).

Table 2: summary of lenticular opacities(expt I)

| day of exam | 1000 ppm | 300 ppm | 200 ppm | 100 ppm | 60 ppm |
|---|---|---|---|---|---|
| 1 | 10/10 neg. | not examd. | not examd. | not examd. | not examd. |
| 2 | 10/10 neg.† | " " | " " | " " | " " |
| 3 | 3/10 p.s.h.<br>1/10 ?p.s.h.<br>3/10 died | 2/10 p.s.h.Bl.<br>2/10 p.s.h.Ul. | " " | " " | " " |
| 4 | 2/7 p.s.h.Bl. (faint)<br>1/7 p.s.h.Ul.<br>3/7 died | 6/10 p.s.h.Bl.<br>2/10 p.s.h.Ul. | 1/10 p.s.h.Ul. | " " | " " |
| 5 | 4/4 died | 2/10 p.s.h.Bl.<br>0/10 p.s.h.Ul. | 7/10 neg.[a]<br>3/10 accidently killed | 10/10 neg. | " " |
| 7 | — | 1/10 p.s.h.Bl. | 7/7 neg. | 10/10 neg. | " " |
| 8 | — | 10/10 neg. | 7/7 neg. | 10/10 neg. | " " |
| 18 | — | 6/10 s.h.Bl.<br>2/10 s.h.Ul.<br>2/10 ?s.h. | 7/7 s.h.Bl. | 10/10 neg. | " " |
| 26 | — | 9/9 s.h.Bl. | 4/6 s.h.Bl | 10/10 neg. | 1/10 e.l.v. |
| 29 | all birds killed except birds on 60 ppm diet with lesions | | | | 4/10 (vacuolar change and/or conjunctivitis |
| 41 | ——— | | | | 2/4 e.l.v. (controls neg.) |
| 45 | ——— | | | | 2/4 vacuolar lens changes (2/5 cont. had similar changes) |
| 48 | ——— | | | | birds killed |

† ophthalmoscopic examination only, slit lamp not used this day; p.s.h.= central posterior subcapsular haziness; Bl= bilateral; Ul= unilateral; s.h.= sutural haziness (mostly posterior); [a] including bird which showed unilateral lenticular change on previous day; e.l.v.= equatorial lenticular vacuolation observed as early as two days of treatment and three days of treatment for 100 ppm (4/10 with s.h., or p.s.h. and s.h.).

In experiment III, to determine a NOEL using lower dietary concentrations (100, 80, 60, 40, and 20 ppm), dinoseb administration for up to four days, resulted in 1/10 bilateral s.h. occurring at day 3 of the study for 80 ppm but not at 4 days on test, i.e., the condition appeared reversible. No effects were reported at 60, 40 or 20 ppm.

CONCLUSIONS/RECOMMENDATIONS:

There was a dose-related increase in lenticular opacities observed in Pekin ducklings administered dinoseb in their diet (1000, 300, 200, 100, 60 ppm) for a period of 26 days or longer( 60 ppm only) which was more apparent as the study progressed. These effects included central posterior subcapsular haziness of the lens, unilateral or bilateral, sutural haziness, and unilateral or bilateral equatorial lenticular vacuolation(e.l.v.) (only observed at 60 ppm and in the controls). The e.l.v. effect in the 60 ppm ducks observed by day 26 was seen prior to a similar phenomenon which was observed in the controls at day 45 but not earlier. This hints at the possibility that dinoseb may have accelerated lenticular changes which may normally occur in the ducks. A similar acceleration of effects noted in controls was seen in a mouse chronic study (J. Rowe; 1986; review of mouse oncogenicity study for dinoseb).

Shorter duration (4 day) studies and lower levels were performed to elucidate the time- and dose-response effect for the lower dose levels used (200 and 100 ppm as well as to establish a NOEL for the study. As the authors noted, these changes "... could be classified as minimal cataractous changes; but, from a clinical standpoint, they are considered to be insignificant regarding their effect on visual acuity." However, in light of the brevity of the study, this statement would appear to be premature. A NOEL for lenticular effects has been established at 60 ppm for a four-day regime but one was not established for a longer term regime (28 days).

This ancillary study is classified as Core Supplementary.

STUDY EVALUATION

W. Tatara
Secondary Reviewer

| | |
|---|---|
| Study Title: | Results of 91-day dietary feeding studies of 2-secondary butyl-4,6-dinitrophenol (DNOSBP) in beagle hounds |
| Reference : | MRID 114962 |
| Testing Facility: | Biochemical Research Laboratory, The Dow Chemical Company |
| Final Report No.: | CDL: 090764-C |
| Final Report Date: | February 28, 1967 |
| Study Authors: | McCollister, D.D. et al. |
| Sponsor: | Dow Chemical Company |
| Test Material: | Dinoseb; 4,6-dinitro-0-sec, butyl phenol (DNSBP); lot # 200206; composition: dinitro-ortho-sec-butyl-phenol 97.5 %, dinitro-para-sec-butyl-phenol 2.1% |
| Dose Levels: | 0, 0.005 and 0.01% DNSBP in dietary feed for 91 days; due to an acceptance problem a fourth group was fed 0.005% for five days, raised to 0.01% for four days, 0.015% for three days and maintained at 0.02% for 79 days; a fifth group was fed 0.02% for 26 days, and increased to 0.03% for 65 days |
| Species: | Beagle hounds (source not stated) |

METHODS:

Groups of male and female dogs (four of each sex/level) were maintained for 91 days on diets containing various amounts of dinoseb (see page 1). The dogs were approximately seven months of age at the start of the dietary study. The stock diet was ground Purina Laboratory Chow mixed with 1% peanut oil. Samples of the prepared dietary levels were analyzed by direct steam distillation followed by visible spectrophotometry of the basic distillate for DNOSBP content. Food consumption records were kept weekly throughout the experimental period. The animals of each sex on each level were caged together and had free access to food and water at all times.

Animals were weighed weekly. They were observed "frequently" for any changes in appearance or behavior. Rectal temperatures were taken twice weekly in an attempt to ascertain indication of metabolic stimulation. Slit lamp examinations for lenticular opacities and cataract syndrome were done periodically by a consulting ophthalmologist.

Hematological values were obtained from all of the dogs before the beginning of the experiment and after 75 days on the diet. Pre-exposure and post-exposure data for serum urea nitrogen and bromosulfophthalein (B.S.P.), serum glutamic oxalacetic transaminase (S.G.O.T.) and serum glutamic pyruvic transaminase (S.G.P.T.) were determined on all dogs. Blood samples were analyzed for DNOSBP content until "the pattern of concentration"(?) was established and then periodically as judged necessary. Standard glucose tolerance tests and urinalysis were done on all of the animals terminally.

Eight dogs (two of each sex per level) from the 0.03 and 0.02 percent levels were put on control feed at the end of the experimental period and maintained for 37 additional days for further observation regarding recovery from dinoseb's toxicity. Additional DNOSBP content of the blood and glucose tolerance tests were conducted on these animals during this period.

At the end of the 91-day test period, the remaining dogs were fasted overnight and weighed before examination at autopsy. Bone marrow smears were taken from the rib of each dog. The lungs, heart, liver, kidneys, spleen, brain, and testes were removed and weighed. Portions of each organ, as well as spinal cord, eye, peripheral nerve, pituitary, thyroid, adrenals, aorta, stomach, small intestine, large intestine, pancreas, gall bladder, urinary bladder, skeletal muscle, ovary, and uterus were placed in formalin. Hematoxylin-eosin stained sections of the tissues were prepared for microscopic examination.

Comments:

1. Significant amounts of dinoseb were observed in the serum of beagles in the controls and all dose levels prior to dietary administration (Table 15 of report, pgs. 31, 32. This was attributed to the previous administration of a drug (not identified) given some time prior for the eradication of hookworm. The ug/ml quantities were: control= 4.7 (average for all animals and for all subsequent data), 0.03%= 4.3, 0.02%=6.5, 0.01%=4.4, 0.005%= 3.9. There were still ug/ml quantities found in the controls at day 83 of the test.

01

2. Ophthalmological examinations were not performed prior to the administration of the compound as recommended in the 1982 EPA Guidelines but rather 5 days into the study.

3. No clinical examination data were submitted.

4. No individual body weight and food consumption data were presented (recommended to be weekly) but rather only the initial and terminal body weights and monthly average (group) food consumption data were given.

5. No measurement of hematological or clinical biochemistry parameters were performed at the midpoint of the study as presently recommended. No measurement of clotting potential or of electrolyte balance, serum albumin, total bilirubin, or total serum proteins was performed.

6. No gross necropsy or histopathology data were submitted. Several organs/tissues were not examined which are currently recommended including: salivary glands, femur with articulation, thymus, parathyroid, bone marrow, esophagus, rectum and representative lymph node.

7. No statistical analyses were performed.

8. A proper satellite group requires separate animals(8:4/sex), not utilization of two/sex from each of the two high dose groups. An inadequate number of animals per dose group was utilized.

RESULTS:

Administration of the two high dose levels (0.03%-.02% for 26 days, 0.03% for remaining 65 days; 0.02 %=0.005% to 0.10% to 0.015% for three to five days at each level (12 days total) and 79 days at 0.020%) resulted in an apparent consistent retardation in body weight increases over the period of the study in both males and females(see growth curves, p. 11 and Table 1 and 2, pgs. 13, 14 of report) as compared to the controls.

The authors stated that there were increases in the average weights of the livers in both males and females at the 0.03% dose level sacrificed at 91 days. However, since there are only two animals/sex for comparison against the control group of four animals this conclusion is questionable. The liver-to-body weight ratio does suggest such an effect but examination of the body weight data indicates unexplained differences in the data which should preclude any conclusion. For example, in Table 1 (p. 12) the terminal weights of male beagles #130 and #164 are given as 9.00 and 8.90 kg, respectively, whereas in Table 12 the final body weight for these same animals are given as 8.70 and 8.20, respectively. Further, the terminal body weights of the female dogs # 163 and 170 are given as 8.65 and 5.80 kg, respectively, whereas in Table 13 the final body weights are stated as 7.60 and 5.40, respectively.

For the 0.03% dose, mural endocarditis, which was "most" striking in the males, was reported. In the males, there was apparent thickening of the endocardium and

the appearance of isolated nests of necrotic fibers in the vicinity of the valves, some of which appeared calcified. The females had some apparent thickening of the endocardium. The livers were reported as enlarged and yellowish in color with microscopic changes of some slight generalized cloudy swelling with loss of liver architecture and some necrosis of hepatic parenchymal cells. For the 0.02% dose, the authors reported mural endocarditis with a small nodule lesion on a valve leaflet in two males with some mural endocarditis seen in the females (number not stated). There was also significant thickening of the endocardium and the appearance of isolated nests of necrotic myocardial fibers in the vicinity of the valves( males only).

In the satellite group, which was maintained for an additional 37 days of recovery after being maintained on a diet of dinoseb for 91 days, there appeared to be a decrease in the liver-to-body weight ratios for both high dose groups as compared to the 91 day controls and the 91 day treated groups. Again, with only 2 animals/sex for each dose are too few for a valid comparison.

The results of examination of the beagles for lenticular opacities are presented below:

Examination of Sept. 12, 1966 (after 5 days of treatment)

Controls: 1 male (#167)-corneal opacity/left eye; 2 females (#s 145, 179)-corneal opacity/left eyes
0.005%: 1 female (#176)-corneal opacity/right eye
0.02%: 1 female (#154)-corneal opacity/left eye

Examination of Nov. 28, 1966 (after 82 days of treatment)

0.01%: 1 male(#157)-corneal opacity(which eye not stated).
0.03%: 2 males (#s 142, 164)- vitreous opacity(left and right eye, respectively).

Previously noted opacities were not reported in the second examination. The authors noted that no vitreous opacity was seen in dog 142 when reexamined on January 9, 1967. The authors did not consider the vitreous opacities observed in the two dogs examined on November 28, 1966 significant, and stated that the opacities were similar to lesions frequently found in humans as a result of trauma but which do not alter vision.

DISCUSSION/RECOMMENDATIONS:

In light of the observation (noted in the methods section) that all animals had been previously exposed to dinoseb, apparently through a treatment of the animals for hookworm, and that significant amounts of dinoseb were found in serum levels prior to commencement of exposure to the test compound, valid conclusions from this study regarding dinoseb's toxicity can not be reached. It should be noted that vitreous opacities were seen toward the end of the study (82 days) in two of the animals, 2 male dogs at 0.03%, and corneal opacity in 1 male dog at 0.01% suggesting that the eye may be a potential target organ for dinoseb--an effect also noted in the Pekin duck. The liver and heart also appear to be affected.

This study is classified as Core Invalid due to the major deficiencies noted in the methods section.

63

STUDY EVALUATION*

Secondary Reviewer

| | |
|---|---|
| Study Title: | Effects of pesticides on the immune response |
| Reference: | MRID # 134602, EPA 600/1-79-039 |
| Testing Facility: | Department of Biochemistry, Scripps Clinic and Research Foundation, La Jolla, California 92037 |
| Final Report No.: | Environ. Sci. Technol., 14:204-210 |
| Final Report Date: | 1980 |
| Study Authors: | Dandliker W.B., et al. |
| Sponsor: | N/A |
| Test Material: | Dinoseb obtained as an analytical standard from EPA; methotrexate obtained from Lederle Laboratories, Pearl River, NY |
| Dose Levels: | Actual dose not stated; single dose, one-half $LD_{50}$ value |
| Species: | Hamsters, inbred males( strain LHC/LAK) |

* This evaluation is based primarily on a review of this study by R. Sjoblad performed on 3/24/86

METHODS:

Dinoseb was one of seven environmental chemicals used in a screen to test for potential effects on: 1) normal delayed type hypersensitivity (DTH) reactions, 2) circulating antibody (serum concentration, heterogeneity, and 3) body weights.

Dinoseb was administered (in 1 ml corn oil) orally in a single dose to hamsters 5-8 weeks old. The actual dose was not reported, but was one-half of the $LD_{50}$ value as reported in "Analytical Reference Standards and Supplemental Data for Pesticides and Other Organic Compounds" (publication no. EPA-600/9-76-012). Numbers of test animals in the treated and control groups were not reported, but for the in vivo DTH studies, was "...usually five animals".

Primary immunization was done by subcutaneous injection, into the flank of each animal, of 0.2 ml of a mixture of 0.1 ml fluorescein isothiocyanate-labeled chicken ovalbumin (FO) and 0.1 ml of complete Freund's adjuvant (CFA). Primary immunizations with FO-CFA were done at 24 hours before dosing with dinoseb. Each animal received 0.4 mg of FO at primary immunization. Booster immunizations were done at 7-day intervals after primary immunization, and consisted of 0.2 ml FO-CFA, with the FO concentration administered at 40 ug per animal.

Blood was drawn by cardiac puncture, and immunoglobulin preparations from pooled serum samples (number of bleedings/pooled serum sample were not reported) were obtained by ammonium sulfate precipitation under alkaline conditions (pH 8.1-8.2). Serum antibodies were evaluated by using a "fluorescence polarization" technique, and the parameters that were measured, as a function of time after primary immunization, included: 1) antibody titer (as a function of antibody concentration and antibody-binding affinity); 2) the number of antibody-combining sites specific for fluorescein; 3) the average association constant of serum antibody against fluorescein; and, 4) an index of the heterogeneity of the binding sites specific for fluorescein.

Body weights were determined at one week intervals over a time period of 5 weeks after dosing with dinoseb.

The known immunosuppressant methotrexate (dose level was not reported) was used to dose animals in the positive control group.

An in vivo test was used to determine the potential for dinoseb to influence a normal DTH response in hamsters. In this assay, test animals were challenged in one footpad with FO at 40 ug. The contralateral footpad, which served as the control for each test animal, was injected with buffer alone. At 24 hours after challenge, reactions at footpads were evaluated by subjective grading of the color and swelling. In addition, thermocouples were used to electronically determine the temperature differences between test and control footpads. Some footpads were removed for histopathological examination. DTH responses were determined at 10, 18, 24, and 31 days after dosing with dinoseb. (Note: the examination dates presented here and elsewhere in this review, are estimates from the bar graph figures provided in the original report.)

Comments:

1. The actual numbers of animals included in each group, and used to generate the data were not provided.

2. Results were presented as data averages in bar graph figures. No raw data, individual animal data, or indices of statistical significance were reported.

3. The methods and techniques used in the analyses were not the most sensitive ones available to use in investigating the effects of dinoseb on normal DTH reactions and serum antibodies.

4. The actual concentrations of dinoseb administered to the test animals were not presented.

RESULTS:

Male hamsters treated with a single oral dose of dinoseb at one-half of the $LD_{50}$ concentration, showed a decreased average body weight when compared to control animals which were treated with vehicle alone. The effect was noticed at the first weighing interval (at one week after dosing) and persisted to the last weighing interval at five weeks after dosing. Data were presented as an average of "percent weight change" versus time. No food consumption data were provided.

Antibody titers, as determined by fluorescence polarization, were depressed in the dinoseb treated group at 21, 35 and 50 days after dosing. When compared to the control group, the dinoseb treated group also exhibited a decrease in the number of antibody-combining sites specific for fluorescein. However, the only time point at which this parameter was evaluated was at 50 days after dosing.

Also, at 50 days after dosing, dinoseb appeared to depress the average association constant of serum antibody against fluorescein; however, the significance of this result could not be determined.

At 10, 18, 24, and 31 days after dosing with dinoseb, the treated hamster group exhibited a reduced footpad DTH reaction to ovalbumin, when evaluated in terms of redness and swelling (on a grading scale of 1-4). The temperature differences between antigen-challenged and control footpads in the dinoseb-dosed group also were consistent with the contention that the test chemical interferes with the normal DTH response.

DISCUSSION/RECOMMENDATIONS:

The validity of this study cannot be determined because individual animal data, raw data and numbers of animals used to generate each point were not reported. This study is considered as a preliminary report. It indicated that dinoseb may interfere with the development of normal DTH responses in hamsters, and also may cause some suppressive effects on circulating antibody in these test animals. Data available from other toxicological investigations with dinoseb should be evaluated closely for determination of any potential immunotoxic effects.

This ancillary study is designated as Core Supplementary data.

005121

STUDY EVALUATION

Secondary Reviewer

Study Title: Testicular effects of dinoseb in rats

Reference: N/A

Testing Facility: Health Effects Research Laboratory, U.S. EPA, RTP, North Carolina 27711

Final Report No.: Arch. Environm. Contam. Toxicol. 11:475-485

Final Report Date: 1982

Study Authors: Linder R.E. et al.

Sponsor: N/A

Test Material: Technical grade dinoseb (97.3%), lot # AGR133942 obtained from Dow Chemical Co.

Dose Levels: Experimental diets of 0, 75, 150, 225, 300 ppm for 11 weeks (77 days)

Species: Rats, adult male, Strain: Sherman

BACKGROUND:

This study is an extension of a study performed by Hall et al.(Tox. Appl. Pcol. 45(1): 235, 236/abstract) which was intended as a combination subchronic feeding and single generation reproduction study. Eight groups of 35- to 38-day old Sherman strain rats (14 of each sex/group) were fed a diet containing nominally 0, 50, 100, 150, 200, 300, 400, and 500 ppm of technical dinoseb (80%) for 60 days and bred, and the parents and offspring continued on study for a total exposure of the parents of 153 days. The 300, 400, and 500 ppm groups were terminated at 21 days due to mortality of 14, 100, and 100%, respectively. Growth in the remaining groups was depressed monotonically at 200, 150, 100, 50 ppm. Liver, spleen, heart, lung and brain weights were decreased while their organ weight /body weight ratios increased. Blood alkaline phosphatase, alanine aminotransferase, potassium, and BUN were significantly increased while LDH and cholinesterase were depressed. Tissue levels were dose dependent with blood>feces>urine>adipose> brain>liver. Aminopyrine N-demethylase activity was increased. Discrimination learning was not affected while locomotor activity was increased at 200 ppm. A significant pathologic change was diffuse tubular atrophy of the testes, noted particularly at 200 ppm. Fertility, fecundity, neonate survival, weight gain, viability and lactation were all depressed.

METHODS:

Technical grade dinoseb was melted in a water bath, mixed to ensure homogeneity, and 6-g aliquots were refrigerated. Each week, an aliquot was thawed, mixed with 12 grams of corn oil, and blended into 432 g of Purina Laboratory Chow® (meal) and diluted with meal in a planetary mixer to obtain a 600 ppm concentrate. The premix was used to prepare the final concentrations. A control diet containing only 0.0% corn oil was used. The diet preparation was assayed for mixing efficacy and also for stability after three days in the food cup and seven days in the storage canister. Samples from the upper and lower portions of the feed containers were analyzed by gas chromatography after hydrolysis with HCl, extraction with ether-hexane, derivatization with acetic anhydride, and subsequent extraction of the acetate derivative with hexane.

Sherman strain rats were provided by the U.S. Center for Disease Control, Atlanta, GA. Adult males, 99 to 115 days of age, were randomly assigned to six treatment groups. Thirty-six rats were fed 0 ppm(controls) and 20 rats each were fed 75, 150, 225 ppm dinoseb, and 36 rats were fed 300 ppm dinoseb. A sixth group of 20 animals were fed control diets and served as a pair-weight control (PW). Except for the PW and 300 ppm groups, which were weighed daily, animals were weighed weekly. Rats in the PW were matched with a partner of similar weight in the 300 ppm group, and food restricted in the PW group so that the weight gain depression was similar in the two groups. The rats were fed their respective diets ad libitum (except PW) and caged 2 per cage (PW caged individually) in suspended wire floor cages equipped with an automatic water system. The animals were housed in a Class 100 Laminar Flow BioClean Room® maintained at 23±3° C with a 12 hr on-off light cycle. Food consumption was measured over a 48-hr period during weeks 1 to 6 and week 9. Colonic temperature was measured (pm) on 5 rats of each group on days 4 and 1 before treatment to accustom the rats to the procedure, then 24 and 48 hrs after providing the diets and periodically thereafter during treatment. Four rats each in groups fed 0 or 300 ppm were sacrificed for terminal studies after 10, 20, 30 and 50 days of treatment. One-half the surviving rats in

each of the six groups were sacrificed for terminal studies during the eleventh week of treatment (71 to 77 days). The remaining rats were used for reproduction studies and subsequent recovery phase terminal studies.

After 77 days of treatment the rats scheduled for breeding were fed regular pellet rations ad libitum. The males were pair-bred with each of two untreated virgin females during the 2-week period following discontinuation of treatment. The presence of a copulatory plug in the cage pan was considered evidence of insemination and the sexes were then separated. Counting the date of insemination as day 0, females were killed on day 20, and implants, fetal viability, and fetal weights recorded. Females with unconfirmed insemination dates were also examined but the fetal weights were not used in tabulating the data. The same males were again bred 104 to 112 days after withdrawal of treatment.

Sperm counts were measured from epididymal fluid using a method similar to the technique of Mason and Thompson (Tox. 8:143, 1977). The caput and corpus portions of the epididymis, testes, seminal vesicles (with coagulating glands), and prostate were weighed, and along with the lungs and bronchi, were fixed in 10% buffered neutral formalin for light microscopic examination.

Body weights, organ weights, sperm counts, and body temperature were subjected to one-way or two-way analysis of variance. Sperm counts of macerated tissue were adjusted for the tissue weight, and organ weights were adjusted for terminal body weight by analysis of covariance. When analysis of variance indicated significant differences between groups, means or adjusted means were compared by Duncan's multiple range test. Sperm morphology profiles were established with cluster analysis (Quesenberry 1964) and group comparisons were made with Fisher's exact test. The Mann-Whitney U test was used to compare resorptions

Comments:

This study was obtained from the open literature and was not intended to meet the regulatory requirements of a reproductive test for which it might be considered.

RESULTS:

Male rat body weights were consistently lower than controls during the treatment period at the two highest doses (300>225 ppm: 3% and 19% average depressed weight gain) with partial recovery at the 300 ppm dose after cessation of dinoseb administration and nearly complete recovery at 225 ppm by day 190 or so. Animal weights for 150 ppm were essentially the same as the controls during the treatment period and, along with the PW animals, completely recovered by the study's end. This recovery is related to increased food consumption which was observed to increase in the 150, 225 and 300 dose groups by the fourth week of dinoseb exposure.

Table 1: reproductive findings in males (11 weeks treatment)
(from table 2, p. 479 of paper)

| mating period | dose level (ppm) | # mated M | # mated F | copulatory plugs | males siring litters | live[a] litrs | fertil.[b] index | fetal observations[c]: average implants | average mortality (%) | fetal weight (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| 0-14 days post-trmt. | 0 | 10 | 20 | 15 | 9 | 16 | 80 | 11.7±.4 | 13.1±3.1 | 3.2±.1 |
| | 75 | 10 | 20 | 12 | 8 | 12 | 60 | 10.1±.9 | 12.5±6.1 | 3.7±.2 |
| | 150 | 10 | 19 | 16 | 9 | 13 | 68 | 11.2±.6 | 9.5±3.0 | 3.3±.1 |
| | 225 | 10 | 20 | 16 | 1 | 2 | 10 | 9.0±.5 | 0 | 3.1±.1 |
| | 300 | 5 | 10 | 2 | 0 | 0 | 0 | 0 | ------ | ------ |
| | PW | 10 | 20 | 5 | 9 | 11(1) | 60 | 10.1±.9 | 17.8±8.4 | 3.9±.5 |
| 104-112 days post-trmt. | 0 | 10 | 10 | 10 | 9 | 9 | 90 | 11.4±1.0 | 23.2±7.0 | 3.5±.1 |
| | 75 | 10 | 10 | 7 | 8 | 8 | 80 | 11.9±1.1 | 17.6±7.9 | 3.4±.1 |
| | 150 | 10 | 10 | 9 | 8 | 7(1) | 80 | 11.4±1.1 | 27.9±11.4 | 3.3±.2 |
| | 225 | 10 | 10 | 7 | 2 | 2 | 20 | 9.0±1.0 | 10.0±10.0 | 3.7±.1 |
| | 300 | 5 | 5 | 3 | 0 | 0 | 0 | 0 | ------ | ------ |
| | PW | 10 | 10 | 5 | 4 | 4 | 40 | 10.5±.7 | 25.8±11.8 | 3.6±.1 |

[a] numbers in () are dead litters; [b] fertility index= litters/matings x 100;
[c] group means±SEM where litter used as unit for calculation

For clinical observations, the animal temperatures were significantly elevated ($p<0.05$) in the 300 ppm and PW groups by 9 days of treatment and in the 225 ppm group by 16 days as compared to the controls. The temperatures were elevated during the remainder of the treatment period. Toxic signs associated with this elevated temperature in the 225 and 300 ppm were weakness, irregular and rapid breathing and the more severely affected animals sometimes salivated profusely. The rats in the PW group were reported more irritable than controls and, except for emaciation, appeared normal. Nine males fed the high dose died between 36-70 days of treatment and one died on the third day of withdrawal. One rat (225 ppm) died on day 57 and one control died on day 59.

Review of Table 1 above indicates males were basically infertile when mated at 0-14 days post-treatment at the 225 and 300 ppm doses. Remating at 104-112 days post-treatment did not result in any substantial increase in fertility. Libido for the 225 ppm group did not appear to be affected as evidenced by the presence of 16/20 females with copulatory plugs whereas it was diminished in the high dose (2/5 females with plugs). A similar effect was observed in the 104-112 day post-treatment matings. Fertility appeared reduced in PW males at the 104-112 day post-treatment matings (40% fertility index) but not in the 0-14 day post-treatment where 9/10 males produced live litters (11/20 females). Although fertility was reduced in the 225 ppm dose group, in females becoming pregnant the number of implants appears to be only slightly reduced and the average mortality or fetal weights were not affected as compared to controls. A similar effect is observed for the 225 ppm dose group at the later mating period. The PW groups were not affected in regards to average implants, average mortality or fetal weight at either mating period.

Table 2: final body weights and selected organ weights (group means±SEM in g)
(from table 3, p. 480 of paper)

| length of treatment | dose | body wt(g) | wt gain(g) | testes | epididymist | seminal vesicles | prostate |
|---|---|---|---|---|---|---|---|
| 71-77 | 0 | 516±13 | 54±6 | 3.6±.12 | 1.48±.05 | 1.25±.10 | .86±.05 |
| | 75 | 505±12 | 48±5 | 3.8±.11 | 1.61±.04 | 1.38±.06 | .97±.06 |
| | 150 | 456±10* | -13±9* | 3.72±.10 | 1.36±.04 | 1.19±.03*** | .82±.07 |
| | 225 | 373±9* | -74±15* | 2.15±.13** | .90±.03** | .82±.05*** | .47±.04 |
| | 300 | 277±6* | -179±17* | 1.20±.19** | .51±.08** | .24±.05** | .16±.05 |
| | PW | 277±9* | -181±11* | 2.62±.19 | .88±.08 | .33±.03 | .21±.04 |
| 77 (16 wk recovery) | 0 | 578±23 | 50±5 | 4.06±13 | 1.63±.06 | 1.64±.05 | 1.01±.06 |
| | 75 | 558±5 | 53±5 | 3.69±.09 | 1.49±.06 | 1.68±.07 | .91±.06 |
| | 150 | 552±12 | 90±5* | 3.57±.28 | 1.51±.07 | 1.79±.10 | .93±.05 |
| | 225 | 519±20* | 132±19* | 2.55±.40** | 1.06±.08** | 1.66±.08 | .80±.05 |
| | 300 | 453±29* | 148±19* | 1.74±.24** | .93±.04** | 1.52±.09 | .62±.07** |
| | PW | 551±15 | 286±14* | 3.92±.11 | 1.52±.04 | 1.66±.06 | 1.02±.04 |

† includes weight of vasa deferentia; * final body weight and weight gain from control significantly($p<0.05$); ** organ weights (adjusted for body weights) significantly less than controls or PW ($p<0.05$); *** organ weights (adjusted for body weights) significantly greater than controls or PW ($p<0.05$)

Table 2 (above) indicates that dinoseb administration induced a significant reduction ($p<0.05$) in body weight and body weight gain at all dose groups of 150 ppm and higher at the end of the treatment period. Statistically significant decreases in the weight of the testes and epididymis were observed at 225 and 300 ppm dose groups, respectively, as compared with controls at 11 weeks. The adjusted seminal vesicle weights were actually greater at 150 and 225 ppm doses than controls while prostate weights were reduced at the two high doses--but not (apparently) statistically significant. At the 11 week period PW body weights and weight gains were significantly reduced and there was some reduction in the testes and epididymal weights with the seminal vesicles and prostate being considerably smaller than the controls. The authors reported that gross pathology showed the testes at 225 and 300 ppm to be small and flaccid while the seminiferous tissue had a liquid consistency and the epididymides were also very small and the surface appeared greyish. The PW groups had small but normal looking prostates and seminal vesicles.

After a 16 week recovery period, the body weight gains remained significantly lower than controls for the two high dose groups while the PW group completely recovered in terms of final body weight and organ weights. In the 225 and 300 ppm groups, testes, epididymides and prostate (300 ppm) weights partially recovered but remained significantly lower than the controls or PW controls. The gross appearance in the two high dose groups was similar to that observed at 11 weeks.

The sperm counts in the epididymis were significantly reduced at 150, 225, and 300 ppm dose levels ($p<0.05$) as well as the sperm cell number in the caudae and vasa deferentia. The sperm cell count in the PW control was significantly lower in the caudae and vasa deferentia but not in the epididymis. In the

Table 3: epididymal sperm counts (group means+SEM)
(from table 4, p. 481 of paper)

| length of treatment | dietary level(ppm) | epididymal fluid[a] # rats | # epididymides | sperm count $10^6$/mg fluid[b] | sperm count of caudae and vasa deferentia # rats | sperm cells ($10^6$) |
|---|---|---|---|---|---|---|
| (71-77) | 0 | 9 | 16 | 1.45±.05 | 9 | 379±40 |
| | 75 | 10 | 19 | 1.57±.07 | 10 | 458±28 |
| | 150 | 10 | 18 | 1.21±.09* | 10 | 206±15** |
| | 225 | 8 | 16 | 0.05±.02* | 9 | 20±4** |
| | 300 | 3 | 5 | 0* | 5 | 9±6* |
| | PW | 9 | 16 | 1.54±.09 | 9 | 173±29* |
| (77) 16 wk recovery | 0 | 10 | 18 | 1.53±.09 | 10 | 369±36 |
| | 75 | 10 | 19 | 1.52±.13 | 10 | 402±60 |
| | 150 | 10 | 19 | 1.55±.09 | 9 | 352±47 |
| | 225 | 5 | 8 | 0.70±.24* | 10 | 78±56** |
| | 300 | 1 | 1 | 0* | 4 | 6±6** |
| | PW | 10 | 19 | 1.50±.06 | 10 | 404±36 |

[a] samples of less than 1 mg excluded; epididymal sample used as unit for calculations; [b] zero values do not indicate azoospermia, only that no sperm cells were present in enumerated squares; * significantly different from control ($p<0.05$); ** significantly different from controls ($p<0.05$) and adjusted count (adjusted for the weight of the caudae and vasa deferentia) differs from control ($p<0.05$)

16 week recovery period the 150 and PW dose groups completely returned to normal sperm cell counts in both the epididymis and caudae and vasa deferentia; the 225 dose group had partial recovery, and the high dose group sperm counts remained unchanged from those observed at the end of the treatment period. The authors noted that decreased sperm counts were observed as early as 30 days at the 300 ppm dose level. Regarding sperm viability, it was noted that no motile sperm were observed in rats fed 225 ppm of dinoseb at the end of the study but motile sperm were seen in 2/10 rats killed after the 16 week recovery period. Motile sperm were observed in only 2/4 rats receiving dinoseb at 300 ppm which were killed on day 20 of treatment. No motile sperm were observed subsequently in any of the 300 ppm rats.

Only 10% of the epididymal sperm cells were classified as normal in the rats fed 300 ppm and killed on day 20 of treatment. The atypical forms at this time were primarily isolated heads--complete cleavage of the head and tail--and incipient separations. In rats killed at 30 and 50 days, many amorphous bizarre forms were also observed including multiple nuclear fragments and multitailed forms which had the appearance of fused cells. Severe oligospermia prevented differential classification at subsequent sacrifices. Many spermatozoa were atypical in the 225 and 150 ppm dose groups at the 11th week sacrifice with the rats (3/5) fed 225 ppm still having abnormal profiles after a 16-week recovery period.

The authors reported that histopathological changes in the testes and caput portion of the epididymides involved primarily the germinal elements. The most severe change was observed in rats fed 300 ppm and killed at 50 days, and in those fed 225 or 300 ppm and killed during the 11th week, in which almost every testi-

cular tubule was involved—necrotic spermatogenic cells and debris, tubules devoid of spermatogenic cells with only Sertoli cells remaining. Minimal changes were reported in the 150 ppm dose group killed at the 11th week with occasional multinucleated spermatogenic cells and abnormal forms of spermatozoa in the testis and/or epididymides. In rats sacrificed after 16 weeks of withdrawal, the 225 and 300 ppm dose groups were essentially the same in terms of histopathological changes as at the end of the treatment period (11th week). Rats fed 300 ppm dinoseb and the PW group, which were killed at the end of the treatment period, showed atrophy of the accessory reproductive glands, particularly the seminal vesicles and coagulating glands. No such effects were observed in any group killed at the end of the 16-week recovery period.

DISCUSSION/RECOMMENDATIONS:

Administration of dinoseb (0, 75, 150, 225, 300 ppm) in the dietary feed of Sherman male rats for an eleven week period followed by a 16-week recovery period resulted in a dose-related effect on body weight, organ weights, mortality, reproductive performance, fetal viability, sperm number in the testes and epididymis, and sperm morphology. Male rat body weights were consistently lower than controls during the treatment period at the two highest doses (300>225 ppm: 38 and 19% average weight losses) with partial recovery at the 300 ppm dose after secession of dinoseb administration and nearly complete recovery at 225 ppm by day 190 or so. An increase in mortality was observed in the high dose group with 9 males dying between 36-70 days of treatment and 1 dying on the third day of withdrawal. The males were basically infertile when mated at 0-14 days post-treatment at the 225 and 300 ppm doses. Remating at 104-112 days post-treatment did not result in any substantial increase in fertility. Statistically significant decreases in the weight of the testes and epididymis were observed in 225 and 300 ppm dose groups, respectively, as compared with controls at 11 weeks. After a 16 week recovery period, the body weight gains remained significantly lower than controls for the two high dose groups while the PW group completely recovered in terms of final body weight and organ weights. In the 225 and 300 ppm groups, testes, epididymides and prostate (300 ppm) weights partially recovered but remained significantly lower than the controls or PW controls.

The sperm counts in the epididymis were significantly reduced at 150, 225, and 300 ppm dose levels ($p<0.05$) as well as the sperm cell number in the caudae and vasa deferentia. The sperm cell count in the PW control was significantly lower in the caudae and vasa deferentia but not in the epididymis. In the 16 week recovery period the 150 and PW dose groups completely returned to normal sperm cell counts in both the epididymis and caudae and vasa deferentia; the 225 dose group had significant but not complete recovery, and the high dose group was essentially unchanged from the end of treatment period. By day 20 of treatment in the 300 ppm dose group only 10% of the epididymal sperm cells were normal looking, and animald sacrificed subsequent to 50 days treatment had oligospermia. Many spermatozoa were atypical in the 225 and 150 ppm dose groups at the 11th week sacrifice with the rats (3/5) fed 225 ppm still having abnormal profiles after a 16-week recovery period. The authors reported that histopathological changes in the testes and caput portion of the epididymides involved primarily the germinal elements.

This study is classified as scientifically acceptable. As stated earlier, this study from the open literature was not intended to fulfill any regulatory requirements.

005421

STUDY EVALUATION

Secondary Reviewer

| | |
|---|---|
| Study Title: | Reproductive toxicity in pseudopregnant and pregnant rats following postimplantational exposure: effects of the herbicide dinoseb |
| Reference: | N/A |
| Testing Facility: | Health Research Center and Department of Biology, Southern University, Baton Rouge, Lousiana 70813 |
| Final Report No.: | Pest. Biochem. Physiol. 18:150-157 |
| Final Report Date: | 1982 |
| Study Authors: | Spencer, F. and Sing, L.T. |
| Sponsor: | EPA Grant R 804974-02 |
| Test Material: | Dinoseb; purity= 95.0%; supplied by Dow Chemical Company (stock # ACR 133942) through the EPA |
| Dose Levels: | Dietary feed with dinoseb in: 1) day 10 pseudopregnant rats fed from days 6 through 9 pseudopregnancy at 0, 25, 50, 100, 200, 250, 350, 500 and 750 ppm or 2) day 16 pregnant rats fed from day 6 through 16 gestation at 0, 50, 100, 150, 200, 250, 300, 350 ppm |
| Species: | Rats, virgin female, Sprague-Dawley |

METHODS:

Rats were propagated in the investigator's laboratory from Holtzman Company shipments. They were individually caged in steel cages and housed under a photoperiod of 14 hr light/10hr darkness at room temperature of 20±2°C. Animals were fed on Purina lab chow and fresh tap water ad libitum. Stages of the estrous cycle were determined microscopically by vaginal smearing. Only those rats (weighing 200-300g) which exhibited two consecutive 4- to 5-day estrous cycles were randomly selected for the experiments.

Pseudopregnancy was induced by stimulating the uterine cervical region of the rat with the introduction of a vibrating fiber glass into the vagina during the proestrus and the estrus stages of the reproductive cycle. Day 1 of pseudopregnancy was designated as the first day when leukocytes appeared predominantly in the vaginal smear. Decidual cell reaction of the uteri was induced by surgical traumatization of both horns at day 4 (1100-1500hr) of pseudopregnancy.

A stock diet of 1.0% dinoseb in a base diet was prepared. From the stock diet, a calculated amount was then taken and further mixed with a given amount of pulverized chow to form the desired dietary concentration of dinoseb. The quantity of food consumption was recorded at the end of every test period.

Dinoseb was fed to the decidualized pseudopregnant rats during days 6-10 of pseudopregnancy which coincides with the period of decidual development. On day 10 of pseudopregnancy, animals were sacrificed by cervical dislocation under light ether anesthesia. The body weights were recorded, the uterine horns removed and weighed to the nearest mg, uterine protein was determined, and uterine glycogen estimated.

Pregnancy was attained by housing a female rat in the estrus stage with a fertile male. The day on which sperm were first observed in the vaginal lavage was considered day 1 of gestation. Feeding of the compound occurred during days 6-15 of gestation which coincides with the duration of an elevated progesterone titer during the advancement of pregnancy (days 5-15) in the rat. The number of implantation sites in the pregnant rat was counted at day 6 of pregnancy, following a tail-vein injection of Chicago blue dye. On day 12 of pregnancy, laparotomy was performed on the same rats on which the implantation sites were previously counted on day 6, and once again the number of developing implantation sites was counted. The percentage of embryo survival rate at day 12 was calculated by the formula: the ratio of the number of surviving embryo per litter at day 12 to the number of implantation sites at day 6(percentage).

On the day (days 20-23) of parturition in pregnant animals carried to term, and whose implantation sites were previously determined, the number of live and dead fetuses was recorded, and the dams were weighed. The fetal survival rate at birth was calculated using the ratio of the number of live fetuses at birth to the number of implantation sites counted at day 6 and expressed in percentage. The live fetuses were examined for external anomalies and weighed. In another experiment, groups of pregnant rats were sacrificed at day 16 of pregnancy and placentae from live fetuses were removed for protein and glycogen analyses.

All data were expressed as mean ±S.E. (standard error). The degree of significance was determined by one-way analysis of variance (ANOVA). The statistical

significance between the experimental and control groups was determined by the Students "t" test with a probability level of $p<0.05$ used as an estimate of significance.

Comments:

This study, obtained from the open literature, was not intended to satisfy the regulatory requirements for a multigeneration reproductive study but to compare the reproductive effects of Dinoseb in pregnant and pseudo-pregnant rats.

RESULTS:

Pseudopregnant

In decidualized pseudopregnant (DCR) studies females were reported to show reductions in body weight gain at all doses tested (25-750 ppm) with the 750 ppm group being significantly different from controls (control=247±6g vs 191±17g). Lethargy and ataxia were also observed at the high dose. Gross necropsy for the 500 and 750 ppm dose groups revealed jaundiced uteri. A statistically significant reduction in absolute and relative uterine wet weight as compared to the controls was observed in the 750 ppm group ($p<0.05$). Uterine protein and uterine glycogen were reduced significantly in a dose-related manner (ANOVA, $p<0.05$) at all dose levels as compared to controls.

Pregnant

In pregnant rats fed dinoseb from days 6 to 15 of gestation, placental protein and placental glycogen concentrations were decreased in 200, 250 and 300 ppm test groups. The investigators noted that, since at higher doses of dinoseb, prominent placental resorption was induced, the placental biochemical parameters were not examined at these dosages. Toxic symptoms, such as ataxia and lethargy, were also observed in all the pregnant rats fed 200 ppm and higher. Gross examination (at day 16 of pregnancy) revealed an accumulation of yellowish fluid in the surrounding fatty tissues of the ovary and the uterus. Dose levels of 150 ppm and higher resulted in statistically significant ($p<0.05$) diminished maternal body weight gain for days 6-12 and days 6-15 of gestation, e.g., days 6-15: 0 ppm= + 38±g vs -30±6 g at 200ppm.

The table below presents reproductive parameters from dinoseb treatment during days 6-15 gestation. No effect on implants from dinoseb treatment were observed at day 6 of gestation. At doses of 200 ppm and higher there was a significant decrease in the number of conceptuses per dam with none observed at 350 ppm dinoseb (ANOVA, $p<0.05$). The percentage of fetal survival rate per litter at birth was significantly reduced at doses of 150 ppm and higher ($p<0.05$). Fetal birth weight per litter (g) was significantly reduced at dose levels of 200 ppm and not determined at the higher doses. The authors noted that a major malformation was observed at 200 ppm with hypoplastic tail formation occurring in 8/62 fetuses examined which was 2/6 litters (33.3%). This anomaly was not observed in the concurrent control or any dose levels of 150 ppm or less.

Table: effect of dinoseb on reproductive performance (days 6 to 15 gestation)
(from Table 2, p. 155 of paper)

| ppm | daily intake (mg/kg/day) | # litter | implants. at day 6 per dam | # conceptuses at day 12 | % embryo surv. rate per litter at day 12[a] | % of fetal surv. rate per litter at birth[b] | fetal birth wt. per litter (g) |
|---|---|---|---|---|---|---|---|
| 0 | ------ | 6 | 12.5±1.4[c] | 12.5±1.4 | 100±00.0 | 80.12±7.59 | 7.20±.30 |
| 50 | 3.26±.09 | 6 | 11.7±1.9 | 11.7±1.9 | 100±00.0 | 83.31±12.56 | 7.13±.27 |
| 100 | 6.90±.20 | 6 | 13.7±1.1 | 13.7±1.1 | 100±00.0 | 63.09±6.05 | 6.78±.14 |
| 150 | 9.23±1.02 | 6 | 14.3±.7 | 14.3±.7 | 100±00.0 | 45.94±11.56* | 6.85±.41 |
| 200 | 10.86±1.33† | 6 | 13.2±1.4 | 9.7±1.1 | 75±9.0* | 53.06±9.20* | 6.43±.18* |
| 250 | 9.38±2/05† | 6 | 12.3±.7 | 7.3±2.6 | 56±22.3* | 16.34±12.32 | ------ |
| 300 | 9.49±1.46† | 6 | 14.8±2.1 | 4.9±3.5 | 33±21.0* | 10.81±5.56* | ------ |
| 350 | 8.60±1.57† | 6 | 12.2±.4 | 0.0±0.0 | 00±00.0 | 00.0±0.00 | ------ |
| ANOVA | | | | p<0.05 | p<0.05 | p<0.05 | p<0.05 |

[a] % age of embryo survival rate= the ratio of the number of surviving embryos per litter at day 12 to that at day 6 as expressed in percentage; [b] % age of fetal survival rate = the ratio of the number of live fetuses at birth to the number of implantation sites counted at day 6 as expressed in % age; [c] mean±S.E.;* significantly different from controls using the Student's t test ($p<0.05$); † the authors stated that no further increase in dose intake was observed for each of these doses, apparently due to ataxia and lethargy in the pregnant rats

DISCUSSION/RECOMMENDATIONS:

Dinoseb administration during days 6-9 in decidualized pseudopregnant (DCR) females produced reductions in body weight at all doses tested (25-750 ppm) with the 750 ppm group being significantly different from controls. Statistically significant reductions in absolute and relative uterine wet weight as compared to the controls were observed in the 750 ppm group ($p<0.05$). Uterine protein and uterine glycogen were reduced significantly in a dose-related manner (ANOVA, $p<0.05$) at all dose levels as compared to controls. This indicates a toxic manifestation of dinoseb on uterine physiology resulting in decrements in the production of uterine protein and glycogen associated with body weight loss. Development of the pseudopregnant uterus is similar to the situation in the pregnant animal in terms of patterns of progesterone and synthesis of a specific protein.

In pregnant rats fed dinoseb from days 6 to 15 of gestation, a parallel effect was observed with placental protein and placental glycogen concentrations decreased in 200, 250 and 300 ppm test groups. Dose levels of 150 ppm and higher resulted in statistically significant ($p<0.05$) diminished maternal body weight gain for days 6-12 and days 6-15 of gestation. At doses of 200 ppm and higher there was a significant decrease in the number of conceptuses per dam with none surviving at 350 ppm dinoseb . The percentage of fetal survival rate per litter at birth was significantly reduced at doses of 150 ppm and higher. Fetal birth weight per litter (g) was significantly reduced at dose levels of 200 ppm and not determined at the higher doses. A major malformation was observed at 200 ppm with hypoplastic tail formation occurring in 8/62 fetuses examined which was 2/6 litters (33.3%). This anomaly was not observed in the concurrent control or any dose levels of 150 ppm or less.

In conclusion, these findings suggest that dinoseb's reproductive toxicity (decreased fetal survival at birth, decreased fetal weight) may be mediated through an effect on the uterine physiology, i.e., the ability of the uterus to adequately support normal fetal development. However, it should be noted that the fetal effects are noted at higher doses ($\geq$150 to 200 ppm) in the pregnant animals as compared to uterine changes (decreased weight, glycogen content) in the pseudopregnant rats which occur at lower doses (25, 50, 100 ppm).

This study is classified as scientifically acceptable. As an ancillary study, it was not intended to meet any of the requirements for studies submitted for the registration of pesticides.

STUDY EVALUATION

Secondary Reviewer

| | |
|---|---|
| Study Title: | Teratology studies in mice with 2-sec-butyl-4,6,-dinitrophenol (Dinoseb) |
| Reference: | MRID # 57711<br>MRID # 39868 |
| Testing Facility: | Department of Pharmacology, Michigan State University, East Lansing, Michigan 48823 |
| Final Report No.: | Food Cosmet. Toxicol. vol. 11, pp.31-43 |
| Final Report Date: | 1973 |
| Study Authors: | Gibson, J.E. |
| Sponsor: | Submitted by Dow Chemical Co. |
| Test Material: | Dinoseb, Dow Chemical Co., lot # 7200206, 1966; DNP(dinitrophenol) from Eastman Kodak Chemicals. |
| Dose Levels: | Dinoseb was administered daily, ip, sc or by oral intubation at various doses (0-50 mg/kg/day): see methods section; dinitrophenol (DNP) was administered in ip doses of 7.7 and 13.6 mg/kg and oral doses of 25.5 and 38.3 mg/kg (equimolar to dinoseb doses of 10, 17.7, 32 and 50 mg/kg, respectively). |
| Species: | Mice, Swiss-Webster strain |

METHODS:

Animals (from Spartan Research Animals, Haslett, Mich.) were housed in groups of ten in stainless-steel cages and allowed food and water ad lib. A 12-hr dark-light cycle was maintained(lights on at 8 a.m.). Pregnant mice were obtained by daily pen breeding from 8 to 9 a.m. (one male/five females). Mice with vaginal plugs were identified as being at day 1 of gestation and were isolated into treatment groups.

Dinoseb was administered to groups of pregnant mice either "throughout" organogenesis*(days 8-16 of gestation), during early organogenesis (days 10-12), or during late organogenesis (days 14-16)--see table below for treatment schedule. The dinoseb doses were selected as those expected by the investigators to produce: 1) maternal toxicity, 2) no maternal toxicity and 3) no embryotoxic or teratogenic effect. Freshly prepared aqueous solutions of dinoseb and DNP (dissolved in 1 N NaOH and titrated to neutral pH with 1 N-HCL) were prepared in concentrations such that 10ml/kg body weight gave the appropriate dose. Control animals received water.

Treatment schedule: Dinoseb

| Dose level (mg/kg/day) | Mortality of dams:#dead/#treated following IP injection | SC injection | Oral intubation |
|---|---|---|---|
| Days 10-12 | | | |
| 0 | 0/8 | 0/7 | 0/6 |
| 10 | 0/11 | 0/7 | --- |
| 12.5 | 0/7 | --- | --- |
| 15.8 | 0/7 | --- | --- |
| 17.7 | 1/14 | 0/7 | --- |
| 18.8 | 5/11 | --- | --- |
| 20 | 4/4 | --- | 0/8 |
| 32 | --- | --- | 0/8 |
| 50 | --- | --- | 6/8 |
| Days 14-16 | | | |
| 0 | 0/7 | 0/8 | --- |
| 10 | --- | 0/3 | --- |
| 12.5 | 0/7 | --- | --- |
| 17.7 | 4/12 | 1/8 | --- |
| 20 | --- | --- | 0/14 |
| 32 | --- | --- | 2/11 |
| Days 8-16 | | | |
| 0 | --- | 0/5 | --- |
| 5 | 0/7 | --- | --- |
| 10 | --- | 0/8 | --- |
| 17.7 | --- | 0/8 | --- |
| 20 | --- | --- | 1/8 |
| 32 | --- | --- | 2/9 |

---not included in test schedule

(*organogenesis is during days 6-15 of gestation)

Caesarian section was performed on day 19 of gestation and the number and position of live, dead and resorbed fetuses were noted. Fetuses were removed by severing the umbilical vessels with a cautery knife to prevent loss of fetal blood, dried on absorbent paper and weighed and the fetal crown-rump distance was measured with a vernier caliper. Individual fetuses were examined for external anomalies, and each litter divided into two sub-groups for fixation in Bouin's solution for 2 weeks or fixed in 95% ethanol and subsequently stained with Alizarin Red S. Tissues from the Bouin's solution were sectioned by hand into 2-3 mm sections and examined under a dissecting microscope for soft-tissue anomalies by the method of Wilson(1965). Skeletons stained with Alizarin Red S were examined for skeletal anomalies.

Statistical analysis of measured parameters was by the analysis of variance (completely random design). Treatment differences were detected by the least significant difference test (Steel and Torrie, 1960). The level of significance was chosen as $p<0.05$.

Comments on methods:

The study was not designed to fulfill the reporting recommendations for a teratology study which would include the following:

1. Justification for the use of mice rather than rats or rabbits.

2. The use of 20 pregnant mice at each dose level with the oral route of administration.

3. The submission of individual data for adults and fetuses including clinical signs, body weights, fetal anomalies, food consumption, etc.

4. The submission of historical data.

5. Establishment of maternal toxicity such as weight loss (highest dose) with not more than 10% maternal deaths as recommended by the 1978, 1982 EPA Guidelines( it is recognized that the study was conducted in 1973 and that this is not a deficiency based on historical consideration but on rather on state-of-the-science considerations).

6. Complete identification of the test substance.

RESULTS:

1. I.P. Injection (methods table, tables 1, 2)

The investigators reported that ip doses of 17.7-20 mg/day of dinoseb produced hyperthermia in the dams with some maternal deaths (45%, 100% for days 10-12 organogenesis at 18.8 and 20 mg/kg/day, respectively; 33% for days 14-16 organogenesis at 17.7 mg/kg/day). Treatment during days 10-12 organogenesis (table 1) produced a decrease in the litter size and an increase in the number of resorptions (statistically significant) at 17.7 mg/kg/day but only a statistically significant reduction in the crown-rump length at the next higher dose(13.8 mg/kg/day) in which there was slightly less than 50% survival of the dams. For days 14-16 of organogenesis, a dose of 12.5 mg/kg/day (7/7 survival) produced a slight reduction in the mean number of fetuses/litter(10 vs 12/cont.) and a statistically significant

Table 1: Fetal data

| dose level (mg/kg/day) | # pregnant mice (treated/surviving) | #implantations†/ # fetuses† | number of resorptions† | fetal b.wt†(g) | crown-rump length(cm)† |
|---|---|---|---|---|---|
| IP | | | | | |
| (days10-12) | | | | | |
| 0 | 8/8 | 13±1/13±1 | 3.5±1.3 | 1.357±.036 | 2.6±0 |
| 10 | 11/11 | 13±1/12±1 | 11.3±4.0 | 1.334±.026 | 2.6±0 |
| 12.5 | 7/7 | 13±1/11±1 | 8.1±2.4 | 1.250±.047 | 2.5±0 |
| 15.8 | 7/7 | 12±1/11±1 | 8.1±2.7 | 1.283±.028 | 2.5±0 |
| 17.7 | 14/13 | 12±0/ 8±1* | 33.9±7.4* | 1.060±.036 | 2.5±0 |
| 18.8 | 11/6 | 13±1/12±1 | 4.5±2.1 | 1.138±.024 | 2.3±0* |
| 20.0 | 4/0 | 0/--- | ---- | ---- | ---- |
| (days14-16) | | | | | |
| 0 | 7/7 | 13±1/12±1 | 8.7±4.5 | 1.355±.030 | 2.5±0 |
| 12.5 | 7/7 | 13±1/10±2 | 36.7±11.1* | 1.169±.036 | 2.5±1 |
| 17.7 | 12/8 | 14±1/ 4±2* | 71.2±11.6* | 1.101±.040* | 2.4±0* |
| (days8-16) | | | | | |
| 5 | 7/7 | 12±1/11±1 | 8.8±3.2 | 1.270±.036 | 2.6±0 |
| SC | | | | | |
| (days10-12) | | | | | |
| 0 | 7/7 | 11±1/11±1 | 4.4±2.2 | 1.339±.032 | 2.6±0 |
| 10 | 7/7 | 14±1/13±1 | 5.2±3.0 | 1.348±.033 | 2.6±0 |
| 17.7 | 7/7 | 13±1/12±1 | 10.0±5.4 | 1.224±.040 | 2.6±0 |
| (days14-16) | | | | | |
| 0 | 8/8 | 13±0/12±0 | 5.6±2.4 | 1.373±.030 | 2.6±0 |
| 10 | 8/8 | 14±1/12±1 | 14.0±6.4 | 1.309±.041 | 2.6±0 |
| 17.7 | 8/7 | 14±1/ 7±2* | 46.4±15.3* | 1.209±.065* | 2.5±0* |
| (days8-16) | | | | | |
| 0 | 5/5 | 13±1/12±1 | 6.4±1.7 | 1.304±.042 | 2.6±0 |
| 10 | 8/8 | 14±1/13±0 | 7.2±2.8 | 1.315±.026 | 2.6±0 |
| 17.7 | 8/8 | 12±1/10±1 | 14.0±7.4 | 1.083±.039 | 2.4±0* |
| Oral | | | | | |
| (days10-12) | | | | | |
| 0 | 6/6 | 10±2/ 9±2 | 4.7±3.3 | 1.315±.042 | 2.6±0 |
| 20 | 8/8 | 11±1/11±1 | 3.5±1.8 | 1.296±.030 | 2.6±0 |
| 32 | 8/8 | 14±1/13±1 | 4.4±1.3 | 1.326±.026 | 2.6±0 |
| 50 | 8/2 | 13±0/12±0 | 4.0±4.0 | 1.268±.039 | 2.5±0 |
| (days14-16) | | | | | |
| 20 | 14/14 | 13±0/11±0 | 8.0±2.7 | 1.261±.022 | 2.5±0 |
| 32 | 11/9 | 12±1/11±0 | 7.4±2.8 | 1.214±.033 | 2.5±0 |
| (days8-16) | | | | | |
| 20 | 8/7 | 14±1/14±1 | 3.1±2.1 | 1.296±.033 | 2.6±0 |
| 32 | 9/7 | 12±1/11±1 | 9.0±2.2 | 1.202±.057 | 2.4±0* |

†mean/litter±SEM; *significantly different from controls($p<0.05$)

increase in the number of resorptions (37%/litter). There was a statistically significant reduction in the mean number of fetuses/litter, fetal body weights, and crown-rump length for a dose of 17.7 mg/kg/day dinoseb at 14-16 days organogenesis (table 1, the number of resorptions was corrspondingly increased).

No effects on any parameter was observed at 5 mg/kg/day during days 8-16 of orga genesis.

Table 2: Gross, soft-tissue and skeletal anomalies from i.p. injection(days10-12

| | INCIDENCE [a] | | | | | |
|---|---|---|---|---|---|---|
| Dose(mg/kg/day): | 0 | 10.0 | 12.5 | 15.8 | 17.7 | 18.8 |
| # Litters: | 8 | 11 | 7 | 7 | 13† | 6 |
| GROSS | | | | | | |
| oligodactyly | 0 | 0 | 1.3±1.3 | 0 | 35.9±9.8* | 15.0±13.1 |
| imperforate anus | 0 | 0 | 0 | 2.9±2.9 | 19.2±8.7* | 6.7±6.7 |
| acaudia | 0 | 0 | 0 | 2.9±2.9 | 17.5±7.7* | 1.7±1.7 |
| microcaudia | 0 | 0 | 0 | 0 | 25.3±8.0* | 7.8±6.5 |
| brachygnathia | 0 | 0 | 0 | 0 | 2.5±2.5 | 0 |
| amelia | 0 | 0 | 0 | 0 | 16.3±8.7* | 0 |
| micromelia | 0 | 0 | 0 | 0 | 5.5±3.9 | 8.3±8.3 |
| open eyes | 0 | 3.6±3.0 | 1.1±1.1 | 8.3±5.9 | 0 | 0 |
| SOFT TISSUE | | | | | | |
| internl.hydroceph. | 14.5±9.4 | 92.0±3.0* | 97.1±2.9* | 55.9±11.5* | 76.2±8.6* | 20.5±5.5 |
| hydronephrosis | 5.4±5.4 | 15.6±4.5 | 23.4±6.3 | 18.9±10.2 | 31.6±8.1* | 18.5±5.7 |
| cleft palate | 1.8±1.8 | 1.3±1.3 | 0 | 0 | 0 | 0 |
| enlarged bladder | 3.6±2.4 | 0 | 0 | 9.4±4.6 | 1.4±1.4 | 8.2±5.3 |
| adrenal agenesis | 0 | 0 | 0 | 0 | 16.2±9.9 | 10.0±10.0 |
| SKELETAL | | | | | | |
| ribs:supernumry. | 27.2±12.2 | 13.1±6.8 | 23.4±13.6 | 20.7±6.1 | 26.0±8.9 | 24.7±5.9 |
| fused | 0 | 0 | 22.1±9.4 | 14.3±14.3 | 54.5±11.6* | 37.2±13.9* |
| absent | 0 | 0 | 0 | 0 | 12.5±7.7* | 0 |
| sternebrae:fused | 0 | 0 | 0 | 0 | 15.6±7.8* | 0 |
| absent and/or not ossifd. | 6.6±4.3 | 11.2±3.6 | 19.7±7.2 | 34.0±13.9 | 56.5±9.8* | 25.8±13.6 |
| vertebrae:fused | 0 | 0 | 4.4±2.9 | 2.0±2.0 | 76.2±8.1* | 37.2±13.9* |
| not ossifd. | 0 | 0 | 0 | 0 | 19.5±8.8* | 0 |
| absent | 0 | 0 | 0 | 7.1±7.1 | 30.8±10.5* | 0 |
| long bones absent or not ossified | 0 | 0 | 4.8±3.1 | 9.1±7.1 | 41.3±10.7* | 13.3±9.9 |

† only 12 litters examined for soft-tissue anomalies; [a] values are the mean percentage responses/litter±SEM; * significantly different from controls ($p<0.05$)

The author only presented anomaly data (table 2) for days 10-12 of organogenesis. It was reported that"...No gross, soft-tissue or skeletal anomalies were detected in foetuses from dams given 5 mg/kg/day ip throughout gestation (d 8-16), nor in foetuses from dams given any dose level on gestational days 14-16. Examination of table 2 indicates a dose-related increase in gross, soft-tissue a skeletal anomalies from doses administered at 10-12 days of organogenesis. At the lowest dose tested(10 mg/kg/ day), there was a significant ($p<0.05$) increase in the incidence of the soft-tissue anomaly internal hydrocephalus (100% dam survival). At a dose of 17.7 mg/kg/day (survival rate of 93%: 13/14) there were numerous significant gross (oligodactyly, imperforate anus, acaudia, microcaudia amelia), soft-tissue (internal hydrocephalus, hydronephrosis) and skeletal fused and absent ribs, fused/absent or not ossified sternebrae, fused/ not ossified/ absent vertebrae, and absent or not ossified long bones) findings. These effect

were considerably diminished in the next higher dose (18.8 mg/kg/day) where dam survival was considerably less( survival rate of 55%:6/11).

Table 3: Skeletal anomalies from subcutaneous (s.c.) or oral intubation of dinoseb during organogenesis

| Dose (mg/kg/day) | # ltrs examd. | Ribs | | Sternebrae | | Vertebrae | | |
|---|---|---|---|---|---|---|---|---|
| | | Supernmry | Fused | Absent or not ossifd. | Fused | Supernmry | Fused | Absent |
| SC(days10-12)[a] | | | | | | | | |
| 0 | 7 | 26.1±10.2 | 0 | 2.4±2.4 | 0 | 0 | 0 | 0 |
| 10 | 7 | 14.3±11.7 | 4.7±4.7 | 22.8±8.4 | 0 | 0 | 0 | 0 |
| 17.7 | 7 | 39.0±8.8 | 33.0±10.7* | 35.6±15.0* | 2.4±2.4 | 6.0±4.0 | 50.3±9.8* | 2.9±2.9 |
| (days14-16) | | | | | | | | |
| 0 | 8 | 20.5±10.8 | 0 | 16.0±5.2 | 0 | 0 | 0 | 0 |
| 10 | 8 | 7.2±4.8 | 0 | 11.6±6.0 | 0 | 1.6±1.6 | 2.5±2.5 | 0 |
| 17.7 | 5 | 19.4±9.8 | 0 | 31.2±9.5 | 0 | 0 | 0 | 0 |
| (days8-16) | | | | | | | | |
| 0 | 5 | 28.0±9.9 | 0 | 2.8±2.8 | 0 | 0 | 0 | 0 |
| 10 | 8 | 44.1±12.0 | 0 | 16.4±5.0 | 0 | 8.0±6.2 | 0 | 0 |
| 17.7 | 8 | 88.4±6.6* | 8.8±4.4 | 39.5±6.6* | 6.6±4.3 | 26.2±11.8* | 7.1±5.4 | 0 |

| | # ltrs examd. | Ribs Supernumerary | Sternebrae Absent or not ossified | Vertebrae Supernumerary |
|---|---|---|---|---|
| Oral (days10-12)[a] | | | | |
| 0 | 6 | 19.0±16.4 | 16.7±16.7 | 0 |
| 20 | 8 | 28.8±11.8 | 16.5±7.1 | 2.5±2.5 |
| 32 | 8 | 6.2±4.4 | 3.5±3.5 | 0 |
| 50 | 2 | 7.0±7.0 | 7.0±7.0 | 0 |
| (days14-16) | | | | |
| 20 | 14 | 7.4±3.8 | 13.2±5.5 | 0 |
| 32 | 9 | 26.9±9.0 | 46.8±10.2* | 0 |
| (days8-16) | | | | |
| 20 | 7 | 65.0±10.3* | 15.9±4.6 | 5.6±2.7 |
| 32 | 7 | 92.1±5.6* | 21.1±11.6 | 42.0±13.7* |

[a] days of gestation on which treatment was given; * significantly different from controls ($p<0.05$)

2. SC Injection (tables 1, 3)

There was essentially 100% dam survival at the doses administered s.c. in the mice(table 1). No fetal effects (decreased fetal survival, fetal body wts., changes in crown-rump lengths) were seen for either 10 or 17.7 mg/kg/day doses of dinoseb administered s.c. at days 10-12 gestation. Statistically significant increases in

in fetal toxicity were produced at 17.7 mg/kg/day for days 14-16 gestation (increased resorptions, decreased fetal body weights and crown-rump length) and days 8-16 gestation (crown-rump length).

SC injection of dinoseb was reported by the author to produce a statistically significant increase in cleft palate only at 17.7 mg/kg/day on days 14-16 of organogenesis (10.6±6.8%:mean %response/litter).

At 10 mg/kg/day an increase (not statistically significant) in absent or not ossified sternebrae (days 10-12; days 8-16) and possibly supernumerary ribs (days 8-16) was produced. At a 17.7 mg/kg/day s.c. injection dosage statistically significant increases in anomalies were observed when administered at days 10-12 (fused ribs, absent or not ossified sternebrae and fused vertebrae) and days 8-16 gestation (supernumerary ribs, absent or not ossified sternebrae and supernumerary vertebrae) but not during days 14-16 gestation.

3. Oral Intubation (tables 1, 3)

Maternal toxicity (mortality) was observed at 20 mg/kg/day (7/8 dams) administered during 8-16 days gestation, 32 mg/kg/day (9/11, 7/9 dams, respectively) during 14-16 and 8-16 days gestation and 50 mg/kg/day (administered only at days 10-12) (table 1). A statistically significant decrease in the crown-rump length for the 32 mg/kg/day dosage given at 8-16 days organogenesis was the only embryotoxicity reported.

No gross or soft-tissue anomalies were reported by the author. No anomalies were produced at any dose level (20, 32, 50 mg/kg/day) with administration during days 10-12 gestation. A significant increase in absent or not ossified sternebrae was observed following administration during days 14-16 of organogenesis, while a dose-related increase (statistically significant) occurred at 20 and 32 mg/kg/day dosages for supernumerary ribs and at 32 mg/kg/day for supernumerary vertebrae during days 8-16 of gestation.

4. DNP (ip or oral) Administration

Dose levels (10, 17.7=ip; 32 and 50=oral; molar equivalents) equivalent to the highest doses of dinoseb used produced overt toxic signs (hyperexcitability and hyperthermia) but were not reported lethal. No morphological defects were reported although there was a statistically significant decrease in mean fetal body weights (1.409±.036 g/cont.; 1.307±.038g) and fetal crown-rump length (2.7±0 cm/cont.; 2.6±0cm) at a dose of 13.6(= to 17.7 dinoseb) mg/kg/day for DNP given ip.

DISCUSSION/RECOMMENDATIONS:

Under the conditions of this bioassay, dinoseb, via ip injection during 10-12 days of gestation, produced dose-related developmental toxic effects for external, soft-tissue and skeletal anomalies. This toxicity appears relative to the production of embryotoxicity, i.e., increased fetal resorptions and decreased fetal body weight. At the lowest dose tested(10 mg/kg/day), there was a significant ($p<0.05$) increase in the incidence of the soft-tissue anomaly internal hydrocephalus (100% dam survival). At a dose of 17.7 mg/kg/day there were numerous significant external (oligodactyly, imperforate anus, acaudia, microcaudia, amelia), soft-tissue (internal hydrocephalus, hydronephrosis) and skeletal(fused

and absent ribs, fused/absent or not ossified sternebrae, fused/not ossified/absent vertebrae, and absent or not ossified long bones) anomalies. These effects were considerably diminished in the next higher dose (18.8mg/kg/day) where dams survival was considerably l[illegible] survival rate of 55%:6/11). The author reported that"...No gross, soft-tissue or skeletal anomalies were detected in foetuses from dams given 5 mg/kg/day ip throughout gestation (day 8-16), nor in foetuses from dams given any dose level on gestational days 14-16."

Route of administration appears to be critical to the production of these defects since subcutaneous injection or oral intubation of dinoseb did not result in the more severe, clearly terata effects such as acaudia or imperforate anus observed with ip injection. The authors did report that sc injection produced a statistically significant increase in cleft palate only at 17.7 mg/kg/day on days 14-16 of organogenesis (10.6±6.8%: mean %response/litter). No severe terata were reported for oral intubation. Sc injection and oral intubation also appeared to be less toxic based on the maternal toxicity observed.

SC injection resulted in an increase in absent or not ossified sternebrae at 10 mg/kg/day (days 10-12; days 8-16) and possibly supernumerary ribs (days 8-16). At a 17.7 mg/kg/day s.c. injection dosage, statistically significant increases in anomalies were observed at days 10-12 (fused ribs, absent or not ossified sternebrae and fused vertebrae) and days 8-16 gestation (supernumerary ribs, absent or not ossified sternebrae and supernumerary vertebrae) but not during days 14-16 gestation.

The effects of oral intubation were relatively milder with no anomalies being produced at any dose level (20, 32, 50 mg/kg/day) with administration during days 10-12 gestation. A significant increase in absent or not ossified sternebrae was observed with oral administration during days 14-16 of organogenesis, while a dose-related increase (statistically significant) occurred at 20 and 32 mg/kg/day dosages for supernumerary ribs and at 32 mg/kg/day for supernumerary vertebrae during days 8-16 of gestation. Decreased c-r length was observed only at 32 mg/kg/day.

DNP was not reported to produce any teratogenic effects at doses equimolar to dinoseb. The author suggests that the active teratogenic constituent of dinoseb is not the parent structure.

In summary, the route of administration appears to be critical to the production of teratogenic or embryotoxic effects since subcutaneous injection or oral intubation of dinoseb did not result in the more severe, clearly terata effects such as acaudia or imperforate anus observed with ip injection.

A NOEL can not be established with any confidence due to the small number of animals tested. As discussed under the methods section this study was not designed for regulatory purposes and is designated as Core Supplementay data.

STUDY EVALUATION

Secondary Reviewer

| | |
|---|---|
| Study Title: | An in vivo/in vitro evaluation of teratogenic action |
| Reference: | N/A |
| Testing Facility: | Department of Anatomy, University of Michigan, Ann Arbor, Michigan 48109 |
| Final Report No.: | Teratology 23:57-61 |
| Final Report Date: | 1981 |
| Study Authors: | Beaudoin, A.R. and Fisher, D.L. |
| Sponsor: | N/A |
| Test Material: | Several compounds including dinoseb which was stated as having been obtained from EPA |
| Dose Levels: | Dinoseb: 10 mg/kg i.p. on day 9, 10 of gestation |
| Species: | Rats, female, Wistar-derived from investigators' colony |

METHODS:

Rats were fed ad libitum with supplemental feedings of lettuce. Water was available at all times. The day when sperm was observed in the vaginal smear was designated as day 0 of pregnancy. Teratogenic doses of the test compounds were administered at 10 AM on day 9 or 10 of gestation.

Twenty-four hours after day 9 injection, or four hours after day 10 injection, the embryos were recovered for culture following the dissection method of New and Coppola ('77). An attempt was made to culture ten embryos from each litter (except only seven from day 10 controls). Some litters, however, had fewer than ten embryos, and some embryos were damaged or lost prior to culture. Usually, therefore, fewer than ten embryos were actually placed in culture. Embryos placed in culture were dorsiflexed, had five to nine somites, a beating heart, and an open neural tube. The culture medium consisted of 50% Waymouth's medium (GIBCO) and 50% fetal calf serum (GIBCO) supplemented with 5,000 units/liter penicillin and 5,000 units/liter streptomycin. A single embryo was placed in a 10-ml screw-cap plastic test tube containing 1.5 ml of medium. Each test tube was gassed with a mixture of 10% $O_2$, 5% $CO_2$, and 85% N, and placed on a rotator and rotated at 30-40 rpm in an incubator at 37° C. At 12, 24, and 36 hours in culture, the $O_2$ concentration was increased to 20%, 50%, and 80%, respectively, with N concomitantly decreased and $CO_2$ maintained constant. Cultivation was terminated at 24 or 42 hours and the development of the embryos evaluated.

After 24 hours in culture, two thirds of the embryos were recovered for examination. The remaining one third was transferred to fresh media and allowed to continue in cultivation until 42 hours. Recovered embryos were examined for rotation of the embryonic axis, heart rate, establishment of the visceral yolk sac circulation , somite number, growth of the limb buds, closure of the neural tube, and development of the allantois and amnion. The results were analyzed by the Student's t-test.

Comments on methods:

At the present time, guidelines for evaluating in vitro teratogenic assays are not available in the Agency, and, hence, a core classification could not be assigned to this study. Scientifically, this study is acceptable. However, it should be noted that this study cannot be used to fulfill the regulatory requirements for a teratology study.

Embryo survival in culture/ embryonic development at 24 hours in culture

[Survival]

| | # pregnant rats treated | | # embryos placed in culture | | survivors at 24 hours[b] (%) | |
|---|---|---|---|---|---|---|
| | day 9 | day 10 | day 9 | day 10 | day 9 | day 10 |
| controls[a] | 41 | 41 | 385 | 271 | 86±10 | |
| dinoseb | 5 | 6 | 38 | 46 | 92±4 | 96±3 |

[a] pooled control values from all experiments; [b] embryos alive at 24 hours and continued in culture were still alive at 42 hours, when the experiment was terminated

[embryonic development at 24 hours in culture]

| | rotated embryonic axis (%) | | closed neural tubes (%) | | anterior limb buds (%) | | no. of somites present | |
|---|---|---|---|---|---|---|---|---|
| | day 9 | day 10 | day 9 | day 10 | day 9 | day 10 | day 9 | day 10 |
| controls | 96±4 | 95±5 | 98±5 | 97±5 | 61±17 | 71±15 | 18±1.0 | 19±0.8 |
| dinoseb | 74±7* | 86±5* | 75±8* | 69±8* | 52±10 | 73±9 | 18±0.7 | 20±0.5 |

*significantly different from the controls ($p<0.05$)

RESULTS/CONCLUSIONS/RECOMMENDATIONS:

The authors noted that since two thirds of the embryos were examined at 24 hours, there were often too few embryos left at 42 hours for a meaningful analysis.

The survival of rat embryos treated at day 9 or day 10 of gestation was comparable to the control embryos. Examination of the cultured embryos at 24 hours indicated that dinoseb significantly inhibited the rotation of the embryonic axis when administered at both day 9 and 10 of gestation. The authors noted that dinoseb still appeared to be inhibitory at 42 hours of culture. In addition, dinoseb significantly delayed the closure of the neural tube in the embryos when they were exposed at either day 9 or 10 of gestation. No effect on the anterior limb bud formation or on the number of somites present was noted.

The author noted that observations made on heart rate, establishment of the yolk sac circulation, and the development of the allantois and amnion did not indicate any consistent pattern of effect for any compound studied, including dinoseb.

It is concluded that dinoseb administration i.p. to pregnant rats at day 9 or 10 of gestation (10 mg/kg) will induce significant inhibition in the development of the embryonic axis and neural tube closure in cultured embryos. This ability to produce "teratogenic" effects is consonant with its effects in vivo. This is a screening test and, as noted in the methods section, is not adequate for regulatory requirements.

005421

STUDY EVALUATION

Secondary Reviewer

| | |
|---|---|
| Study Title: | Postnatal morphology and functional capacity of the kidney following prenatal treatment with dinoseb in rats |
| Reference: | N/A |
| Testing Facility: | Department of Phamacology and Toxicology and Department of Pathology, Michigan State University, East Lansing, MI |
| Final Report No.: | J. Toxicol. Environ. Hlth. 6:633-643 |
| Final Report Date: | 1980 |
| Study Authors: | McCormack, K.M. et al |
| Sponsor: | N/A |
| Test Material: | Dinoseb: Dow Chemical Co., Midland, MI; lot 7200206, 1966 |
| Dose Levels: | I.p. injections (5 ml) of 0, 6.3, 9.0, 11.2, 12.5 and 15 mg/kg on days 10-12 of gestation |
| Species: | Rats, female, strain Sprague-Dawley from Spartan Farms, Haslett, Mich. |

METHODS:

Timed-pregnant rats were obtained on day 2 of gestation. The rats received ip injections of dinoseb (dissolved in 1 N NaOH and titrated to pH 7.4 with 1 N HCl) on gestational days 10-12 and saline (control) in 5 ml volume per kg body weight. Litters were normalized to 5 males and 5 females on day 1 postpartum. Experiments were conducted with offspring at 21 days of gestation or at 1, 7, or 42 days postpartum.

Fetuses used at 21 days of gestation were removed by caesarian section. Numbers and positions of live, dead, and resorbed fetuses were recorded. Fetuses were weighed, measured for crown-rump length with a vernier caliper, sexed, and examined under a dissecting microscope for external anomalies. Offspring were also examined under a dissecting microscopic for visceral anomalies at gestation day 21 (at least 6 litters from the 0, 6.3, 8.0 and 9.0 mg/kg/day treatment groups) as well as at 1, 7, and 42 days postpartum (at least 8 animals per age in the 0, 8.0, and 9.0 mg/kg/day treatment groups). Tissues from offspring at 21 days of gestation (liver, kidney, ureter, heart, and lung) and at 1 and 42 days postpartum (liver, kidney, and ureter) were used for histological examinations. Samples (obtained from at least 3 animals per age in the 0, 8.0, and 9.0 mg/kg/day treatment groups) were fixed in 10% buffered formalin. After fixation, these were embedded in paraffin, sectioned at 5 um, and stained with hematoxylin and eosin.

Renal function of rats treated prenatally with dinoseb (0 or 9.0 mg/kg/day) was determined both in vivo and in vitro. Organic ion transport capacity was quantified as the ability of renal cortical slices to accumulate a representative anion, PAH, and cation, N-methyl-nicotinamide (NMN) by the method of Cross and Taggart (1950). Postnatal renal function was also assessed by quantifying the clearance of inulin and PAH, the BUN, and the maximal urine osmolality.

Data were analyzed statistically by analysis of variance, completely random design. Treatment differences were detected by the least significance difference test (Steel and Torrie, 1960). The 0.05 level of probability was used as the criterion of significance.

Comments

This study, obtained from the open literature, is intended as a teratology screening technique and, at the present time, is useful only as a qualitative indicator of teratogenicity. The test system has not been adequately validated to allow it's use for regulatory purposes.

RESULTS:

In a range-finding study i.p. administration of dinoseb (0, 6.3, 8.0, 9.0, 11.2, 12.5 and 15.8 mg/kg.d) on day 10-12 of gestation resulted in 100% mortality at doses of 11.2 mg/kg/d and greater within 1 week of treatment with some mortality (3/16=20%) at 9.0 mg/kg/d.

Table 1: Resorption rate and size of fetuses from pregnant rats[a]

| Dose | # live fetuses | resorbed or dead (%) | fetal body weight (g) | fetal crown-rump length (cm) |
|---|---|---|---|---|
| 0 | 13±1 | 5.8±2.3 | 5.82±.08 | 3.9±.1 |
| 6.3 | 11±1 | 8.6±3.7 | 5.91±.10 | 3.9±.1 |
| 8.0 | 12±1 | 4.9±1.5 | 5.44±.06[b] | 3.9±.1 |
| 9.0 | 11±1 | 7.1±2.2 | 4.96±.07[b] | 3.4±.2[b] |

[a] values are the mean per litter±SEM for at least 6 litters, rats killed at day 21; [b] significantly different from control value, $p<0.05$

Table 2: Body weight, liver weight/body weight ratio, and kidney weight/body weight ratio[a] (female pups)

| dose (mg/kg/d) | age(d) | body weight (g) | liver wt/bwt (x100) | kidney wt/bwt(x100) |
|---|---|---|---|---|
| 0.0 | 1 | 6.7±.1 | 5.48±.15 | 0.91±.02 |
| 9.0 | 1 | 5.9±.2[b] | 5.63±.21 | 0.93±.02 |
| 0.0 | 7 | 15.6±.4 | 2.97±.04 | 1.10±.02 |
| 9.0 | 7 | 13.8±.6[b] | 3.10±.09 | 1.16±.03 |
| 0.0 | 42[c] | 143.5±9.1 | 4.58±.23 | 0.93±.03 |
| 9.0 | 42[c] | 139.4±7.7 | 5.04±.18 | 0.95±.05 |

[a]values are means±SEM for at least 8 female rats; [b] significantly different from control value, $p<0.05$; [c]values are for females, body weights were 171.8±12.4 and 174.9±18.6 for control and dinoseb-treated male rats, respectively

Dinoseb administration (see Table 1 above) did not result in any increase in the per cent resorbed or dead fetuses at dosage levels of 6.3, 8.0 and 9.0 mg/kg/d but did produce a significant decrease in mean fetal body weights ($p<0.05$) at the two higher doses as well as a decrease at the highest dose (9.0 mg/kg/d) in the mean fetal crown-rump length ($p<0.05$).

Administration of 9.0 mg/kg/d of dinoseb (see Table 2 above) significantly diminished female pup body weights at 1 and 7 days postpartum ($p<0.05$) but this weight reduction was not observed by 42 days postpartum. No significant effect of dinoseb administration during gestation was observed for either liver weight- or kidney weight-to body weight ratios at any day after birth. However, hepatic and kidney histopathologic changes were reported for the 8.0 and 9.0 mg/kg/d doses. In the liver, fetuses (near term) had cell vacuolations that displaced the nucleus and most of the cytoplasm while livers of pups at 42 days postpartum were also vacuolated and necrotic. Other effects included disruption of hepatocytes, absence of nuclei, pyknotic or karyorrhetic nuclei, and cellular swelling with lesions most extensive at the periphery of the lobules. For the kidneys, approximately 40% of near-term fetuses at the same dosages had dilated renal pelves and/or ureters upon gross examination. Histological examination revealed dilation of the renal pelvis and tubules (particularly distal tubules and collecting ducts), prevalence of mesenchymal tissue, and vacuolation of the transitional epithelelium of ureters (near-term and 1 day-old rats). The authors reported

Table 3: accumulation of PAH and NMN by renal cortical slices (pre-natal treatment with dinoseb)[a]

| dose (mg/kg.d) | age (d) | slice-to-medium ratio PAH | NMN |
|---|---|---|---|
| 0.0 | 1 | 3.49±.46 | 2.93±.31 |
| 9.0 | 1 | 2.85±.52 | 2.70±.43 |
| 0.0 | 42[b] | 9.61±1.34 | 6.15±.84 |
| 9.0 | 42[b] | 8.98±1.19 | 6.06±.45 |

[a] values are means±SEM for 8 animals; [b]values are for male rats

that the incidence and severity of lesions in kidneys and ureters decreased with age.

Pre-natal treatment with dinoseb (see Table 3 above) had no effect on the accumulation of PAH or NMN by renal cortical slices from tissues of animals at day 1 or day 42 postpartum. The clearance of inulin (measure of the glomerular filtration rate) and PAH (a measure of the effective renal plasma flow) from day 42 post-partum males was not affected by dinoseb administration. Na and K(uEq/h) excretion, urine osmolality (mOsm/kg $H_2O$) and urine flow (ml/h) in normopenic and hydropenic (48 hr water deprivation, sc injection of ADH) 42 day post-partum females was not affected by dinoseb administration (pre-natal). The authors also reported that BUN determinations in the 42-day-old females were not affected by dinoseb.

DISCUSSION/RECOMMENDATIONS:

Pre-natal administration (i.p./8.0 and 9.0 mg/kg/day) of dinoseb (day 10-12 of gestation) resulted in the occurrence of post-partum effects including decreased fetal body weights (day 1, 7 but not at day 42), a decrease at the highest dose (9.0 mg/kg/d) in the mean fetal crown-rump length ($p<0.05$) in near-term fetuses (day 21), and hepatic and kidney gross and histological changes. In the liver, fetuses (near term) had cell vacuolations that displaced the nucleus and most of the cytoplasm while livers of pups at 42 days postpartum were also vacuolated and necrotic. Other effects included disruption of hepatocytes, absence of nuclei, pyknotic or karyorrhetic nuclei, and cellular swelling with lesions most extensive at the periphery of the lobules. For the kidneys, approximately 40% of near-term fetuses at the same dosages had dilated renal pelves and/or ureters upon gross examination. Histological examination revealed dilation of the renal pelvis and tubules (particularly distal tubules and collecting ducts), prevalence of mesenchymal tissue, and vacuolation of the transitional epithelelium of ureters (near-term and 1 day-old rats). The incidence and severity of lesions in kidneys and ureters decreased with age (42 days).

This study is classified as Core Supplementary since it was intended as a teratology screening technique and is not acceptable for regulatory requirements.

STUDY EVALUATION

Secondary Reviewer

| | |
|---|---|
| Study Title: | The effect of acute maternal toxicity on fetal development in the mouse |
| Reference: | N/A |
| Testing Facility: | Perinatal Toxicology Branch, Developmental Biology Division, HERL, USEPA, RTP, NC |
| Final Report No.: | Teratogen. Carcinogen. Mutagen. 5:3-13 |
| Final Report Date: | 1985 |
| Study Authors: | Kavlock, R.J. et al |
| Sponsor: | N/A |
| Test Material: | Ten chemicals including technical grade dinoseb, Lot AGR 133942, purity 97% from Dow Chemical Co. (Midland,MI) |
| Dose Levels: | 0, 26, 33 mg/kg on day 8 of gestation |
| Species: | Mice, female, random-breed CD-1 from Charles River Breeding Laboratory |

METHODS:

Nulliparous mice (29-33g) were used in the toxicity studies, while primiparous animals received on day 2 of pregnancy(the morning of confirmation of the presence of a vaginal plug being day 1) were used in the teratology studies. Animals were received in weekly blocks during the course of the experiment, with each block representing the complete toxicity or teratology of a particular compound. Each block contained an appropriate concurrent control. Within a block, animals were randomly assigned to one of the available treatment groups.

Compounds were administered orally (intubations) as solutions in corn oil. Intubation volumes were 0.5 ml/mouse.

For toxicity studies, initial doses selected were based on the acute LD50 values from the literature or from extrapolation of sub-chronic toxicity studies done in the investigators' lab. Five groups of ten animals each were assigned to dose groups bracketing the anticipated LD50 value. Animals were observed for death and other signs of toxicity for 10 days following dosing. Probit analysis was used to calculate a dose that would induce a low degree of maternal lethality (the predicted LD10) and one that would induce a moderate degree of maternal lethality (LD40) for use in the teratology studies.

On day 8 of gestation, primiparous females were weighed and dosed with either the vehicle (20 animals) or a dose that induced a low degree of maternal lethality (20 animals) or a moderate degree of lethality (40 animals). Animals were killed on day 18 of gestation, the uterus was removed and weighed. Maternal weight was calculated as the difference between the overall weight gain during pregnancy and the gravid uterus weight. The fetuses were removed from the uterus, blotted and weighed as a group, examined for gross malformations, and divided equally for fixation in either a solution of 5% formaldehyde, 5% glacial acetic acid, and 70% alcohol or in 70% alcohol alone. The former were necropsied, while the latter were stained with alizarin red S and examined for skeletal abnormalities and maturity. Ammonium sulfide staining of the uteri from apparently non-pregnant females was not done, and it is possible that resorptions of entire litters that occurred shortly after treatment may have been overlooked. However, the investigators noted that this particular response would have manifest itself in the % of treated females which survived to the scheduled sacrifice that were "non-pregnant."

The litter was regarded as the fundamental unit of comparison in statistical procedures. For continuous variables, analysis of variance procedures available in the Statistical Analysis System were used to detect overall treatment effects; post hoc t-tests were used to compare individual treatment groups when there was a significant F value in the analysis of variance (ANOVA).

Table 1: maternal effects at low and moderately toxic dose levels

| dose (mg/kg) | # treated | #(%) maternal deaths | #(%) whole litter resorptions | #(%) viable litters[a] | initial maternal wt.(g)[b] | maternal weight gain(g)[b] |
|---|---|---|---|---|---|---|
| 0 | 15 | 0(0) | 0(0) | 12(80) | 28.0±.3[c] | 4.3±.8 |
| 26 | 20 | 0(0) | 0(0) | 17(85) | 27.7±.4 | 4.6±.3 |
| 33 | 40 | 2(5) | 1(3) | 20(53) | 27.6±.3 | 3.6±.5 |

[a] percentage of those females surviving to term; [b] includes only those females that had fetuses at term; [c] mean±standard error of the mean

Table 2: fetal effects at low and moderately toxic dose levels

| (mg/kg) | # litters[a] | % prenatal mortality | weight(g) | # sternal ossifctns. | # caudal ossifctns. | # enlarged cerebral ventricles | % enlarged renal pelvis |
|---|---|---|---|---|---|---|---|
| 0 | 11 | 5±2* | 1.00±.04 | 5.4±.5 | 3.9±.4 | 4±3 | 6±4 |
| 26 | 17 | 6±2 | 1.03±.01 | 6.0±.1 | 4.2±.1 | 4±2 | 0±0[b] |
| 33 | 20 | 4±1 | 0.98±.02 | 6.0±.1 | 4.1±.1 | 0±0 | 1±1[b] |

[a] includes only litters with live fetuses at term; [b] significantly different from concurrent control value, $p<0.05$;*data presented as mean±standard error of the mean

Table 3: malformations in fetuses from dams exposed to low or moderately toxic doses

| dose (mg/kg) | # fetuses/ litters exmd. | enceph- alocele | exence- phaly | miscelln. cranial def. | cleft palate | fused vrtbr. | umbilical hernia | hydrone- phrosis |
|---|---|---|---|---|---|---|---|---|
| 0 | 1,682/156[a] | 3/2[b] | 2/2 | --- | 2/1 | 1/1 | --- | 3/3 |
| 26 | 181/17 | --- | 1/1 | --- | --- | --- | 1/1 | --- |
| 33 | 223/20 | 3/2 | --- | 1/1 | --- | --- | --- | --- |

(Table 3 cont.)

| dose (mg/kg) | short tail | % fetuses/litters with defects |
|---|---|---|
| 0 | 1/1 | 1/6 |
| 26 | --- | 1/12 |
| 33 | --- | 2/15 |

[a] # fetuses affected/# litters affected; [b] due to the low incidence of defects in the various concurrent control groups, the control data were pooled across all groups

005121

STUDY EVALUATION

Secondary Reviewer

| | |
|---|---|
| Study Title: | An extended evaluation on an in vivo teratology screen utilizing postnatal growth and viability in the mouse |
| Reference: | N/A |
| Testing Facility: | Reproductive and Perinatal Toxicology Branches, Developmental Biology Division, HERL, US EPA, MD-72, RTP, NC |
| Final Report No.: | Teratogen. Carcin. Mutagen. 4:403-426 |
| Final Report Date: | 1984 |
| Study Authors: | Gray, L.E.,Jr. and Kavlock, R.J. |
| Sponsor: | N/A |
| Test Material: | 35 different compounds including dinoseb which was stated as being at least 97% pure. |
| Dose Levels: | For dinoseb, 0, 15 and 100 mg/kg/day during days 8-12 of gestation |
| Species: | Mice, female, strain CD-1 from Charles River Breeding Laboratories (MA) |

METHODS:

The present study is an extension of the study presented by Chernoff and Kavlock(J. Toxicol. Environ. Hlth. 10:541-550, 1982). Additional treatments were administered later in gestation than the period utilized by Chernoff and Kavlock to evaluate their hypothesis that the duration of exposure could be altered and the assay still be valid.

In the present study, more than 1500 pregnant mice were exposed to one of 35 different chemicals in 41 different treatment regimens. The dams were allowed to deliver and the postnatal viability, growth, morphology, and reproductive function of the offspring were assessed. The study was conducted over a 2-year period in 21 blocks with two or three treatment groups and a concurrent control per block. Within each block, each treatment group initially contained 23-40 primiparous 90-day-old female mice received on day 5 of gestation. Dams were housed individually in transparent plastic cages with pine shavings as bedding. Animals were maintained on a 12/12 photoperiod with lights off at 1800(EST), with tap water ad libitum, and room temperature of 20-24°C. Upon arrival, dams were randomly assigned to control or treated groups. The treatments were usually administered daily on days 8-12 of gestation which is within the period of major embryonic organogenesis. Doses were selected from published studies or from studies in the laboratory that determined a MTD.

The females were weighed throughout dosing and on day 19 of gestation. They were allowed to give birth and the litters were counted and weighed at 1 and 3 days of age. Dead pups recovered from the cages were necropsied and abnormalities recorded. Dams that had not given birth by postnatal day 3 were killed and examined for the presence of resorptions.

Random samples of the dams and pups were selected from the Chernoff and Kavlock study for observation in the present study. For dinoseb (1st block), litters were randomly selected. At 22 days of age a male and female from each litter were weighed after behavioral testing as were two males from each litter at 57 days. Behavioral data were not presented in the present report. At 30 days of age the pups were weaned, counted, and weighed and the numbers of females with patent vaginas determined. The pups remained in bisexual groups after weaning for breeding purposes. Throughout the remainder of the experiment the animals were checked while the cages were being changed and obviously pregnant animals were removed and individually housed until parturition when litter size and age of the dam were recorded.

At approximately 250 days of age the males were necropsied and body weight and weights of the liver, testes, seminal vesicles, and right kidney were recorded. Any gross pathology was recorded. In the first 19 blocks, survival rates after weaning were calculated from the numbers of females alive through day 60 divided by the numbers of females weaned. The body weight data on day 30 was collected and analyzed by univariate analysis of variance (ANOVA) using the litter means. All other data were analyzed using individual values rather than litter means because, with the exception of block 1, the pups were not genetic littermates. The necropsy data were analyzed by block using a multivariate ANOVA. The data on age at parturition and $F_1$ litter sizes were also analyzed by block using multivariate ANOVA. The percentage data were analyzed by computing a normal deviate z, derived from the normal approximation to the binomial. The z and chi-

square tests are identical. In addition, the control data were analyzed to determine if any of the variables exhibited significant block effects. Correlation matrices were calculated for 1) the control groups, 2) the compounds that are known to cause frank malformations or resorptions, and 3) the compounds that do not cause malformations or resorptions using group means of the maternal and neonatal data from the Chernoff-Kavlock Assay(CKA) and data from this study.

Comments

This study was obtained from the open literature and was intended as a teratology screening technique. At the present time, it is useful only as a qualitative indicator of teratogenicity. The test system has not been adequately validated to allow it's use for regulatory purposes.

RESULTS:

As Table 1 below illustrates, prenatal exposure of CD-1 female mice (day 8-12 of gestation) to dinoseb at a MTD produced no significant effects on any of the postnatal parameters studied including the number of pups (males and females) alive at day 3, the body weight at day 3 (both sexes), and body weights at day 22 or 30 (both sexes) or day 57 (males). No decreases were observed in body or organ weights (liver, testes, seminal vesicle, kidney) at day 250 when necropsy occurred.

In dinoseb treated animals a 57% vaginal patency at day 30 in female mice was reported as opposed to only 24% in the concurrent controls, however other concurrent controls had patencies ranging from 29 to 100%.

Table 1: effects of prenatal exposure to chemicals on the postnatal growth and organ weights of CD-1 mice[a]

| dose: (mg/kg/d)/ route | day3 #live (M+F) | day3 b.wt. (M+F) | day 22 body wt. (M-F) | d30/d57 body wt. M | d30/d57 body wt. F | weights at necropsy body (M) | liver (M) | testes (M) | sem.ves. (M) | kidney (M) |
|---|---|---|---|---|---|---|---|---|---|---|
| control 0/oral | 9.1 | 1.58 | 8.3 | 22.1/ 30.2 | 19.3 ---- | 43.2 | 2.485 | 226 | 550 | 369 |
| dinoseb 15/oral | 9.9 | 2.00 | 8.5 | 22.7 29.9 | 20.3 ---- | 42.1 | 2.264 | 269 | 543 | 382 |

[a] dams were dosed on days 8-12 of gestation; males were necropsied at day 250

DISCUSSION/RECOMMENDATIONS:

This study was intended as an extended evaluation of the study performed by Chernoff and Kavlock (1981) in a proposed teratology screen. Prenatal exposure of CD-1 female mice (day 8-12 of gestation) to dinoseb at a MTD produced no significant effects on any of the postnatal parameters studied including the number of pups (males and females) alive at day 3, the body weight at day 3 (both sexes), and body weights at day 22 or 30 (both sexes) or day 57 (males). No decreases were observed in body or organ weights (liver, testes, seminal vesicle, kidney) at day 250 when necropsy occurred.

This study is classified as Core Supplementary due to its use as a teratology screen and not as a complete teratology test.

005421

STUDY EVALUATION

Secondary Reviewer

Study Title: An in vivo teratology screen utilizing pregnant mice*

Reference: N/A

Testing Facility: Developmental Biology Division, Health Effects Research Laboratory, U.S. EPA, Research Triangle Park,NC 27711

Final Report No.: J. Toxicol. Environ. Hlth. 10:541-550

Final Report Date: 1982

Study Authors: Chernoff, N., and Kavlock, R.J.

Sponsor: N/A

Test Material: Dinoseb and 27 other compounds

Dose Levels: 15 mg/kg via oral intubation (dinoseb) on day 8-12 of gestation

Species: Mice, female of CD-1 strain (Charles River Laboratories, Mass.)

* Also presented in Environ. Sci. Res. 27:417-427, 1983 as "A teratology test system which utilizes postnatal growth and viability in the mouse"

METHODS:

Animals were individually housed in solid-bottom cages with wood shavings for bedding, kept in temperature-controlled (20-24°) bio-clean rooms with a 12-hr light/dark cycle, and were fed commercial lab diet and water ad lib.

The minimally toxic dose levels (MTD), were determined in nonpregnant mice housed five/cage. Compounds were administered on a mg/kg basis (0.5 mL/d) for 5 consecutive days at one of five dose levels. Dinoseb was administered by oral gavage. Each dose level consisted of 10 animals. The MTD was considered to be that dose resulting in either significant weight reduction during the treatment period, mortality, or other signs of toxicity.

In the definitive studies, pregnant mice were singly housed and received one of a variety of chemicals (28) including dinoseb, by either gavage (0.5 mL/d) or intraperitoneal injection (0.2 mL/d). The route of administration reflected that route used previously to demonstrate the teratogenic potential of the compound. Animals were dosed on d 8-12 (or single days within that period), within the period of major organogenesis. Compounds were administered at or near the MTD level. A treated group generally consisted of 24-30 dams and the control group contained 24-40. The change in maternal weight during the treatment period was calculated. Dams were allowed to give birth, and the litters were counted and weighed on postnatal day 1 and 3 (PD1 and PD3). Mice in the facility normally delivered on the evening of day 19 of gestation, and day 20 of gestation was therefore defined as PD1. Dead pups recovered from the nest were necropsied and abnormalities noted. Dams not giving birth by PD3 were killed and their uteri examined for the presence of implantation sites.

All data analyses compared treatment groups and their concurrent controls and were performed using analysis of variance (ANOVA) procedures available in the General Linear Model Procedure on version 79.2B of the Statistical Analysis System (SAS User's Guide). When a significant treatment effect was detected by ANOVA, individual group means were compared using Student's t test on least square means. Since an a priori hypothesis was that treatments would only reduce litter size, one-tailed tests were used for the analysis of the number of live pups on day 1 and 3. To correct for differences in pup weights due to differing litter sizes, the number of live pups on day 1 was used as a covariate in the analysis of postnatal body weights. Dams that had nidation sites but no record of having given birth were considered to have no live pups on PD1 and PD3.

Comments

This study was obtained from the open literature and was intended as a teratology screening technique. The test system has not been adequately validated to allow it's use for regulatory purposes.

RESULTS:

Results of the screen are given below:

Maternal and perinatal effects of prenatal administration of dinoseb[a]

| treatment regimen[b]: dose(mg/kg)/route/ vehicle/days | # treated/ # died | # pregnant | maternal wt.change | # alive[d] average wt. d 1/d 1 | d 1/d 3 (gm) |
|---|---|---|---|---|---|
| control oral/co/8-12 | 24/0 | 19 | 6.5±.4 [e] | 10.2±.6/ 9.1±.4 | 1.53±.03/ 1.49±.05 |
| dinoseb 15/oral/co/8-12 | 23/1 | 12 | 6.8±.5[c] | 10.1±.4/ 9.3±.3 | 1.54±.04/ 2.00±.10 |

[a]values are mean ± SE ; [b] corn oil; [c] % of concurrent control; [d] number per litter; [e] per cent of the total mean control data used in all treatments (sum of concurrent controls)

There was a 79% fertility rate in the concurrent control versus 52% in the dinoseb treated animals which reduced the sensitivity of the screen to detect effects on litter size. Dinoseb produced no statistically significant changes in maternal weight, average number (per litter) alive at day 1 or 3 postnatal, and average weight changes in the pups (per litter) at days 1 and 3 after birth. The authors concluded that the screening test for all the compounds, including dinoseb, correlated with the results obtained from standard teratology tests in the mouse.

The authors concluded that oral gavage of dinoseb (during day 8-12 of gestation) in this teratology screening test did correlate with its lack of developmental toxicity in the mouse (as measured by a standard teratology test: study not cited) as measured by the lack of effects on litter size, maternal weight changes, and pup viability or weight measured at day 1 and 3 postnatally. (It should be noted, however, that Dinoseb has been shown to have developmental toxicity, particularly by the i.p. route of administration in a number of studies). As a teratology screen this study does not meet the regulatory requirements for a teratology test. This study is considered as Core Supplementary data.

005421

STUDY EVALUATION

Secondary Reviewer

Study Title: Effect of food deprivation, phenobarbital, and SKF-525A on teratogenicity induced by 2-sec-butyl-4,6-dinitrophenol (dinoseb) and on disposition of [$^{14}$C]dinoseb in mice

Reference: N/A

Testing Facility: Department of Pharmacology, Michigan State University, East Lansing, Michigan

Final Report No.: J. Toxicol. and Environ. Health, 1:107-118

Final Report Date: 1975

Study Authors: Preache M.M. and Gibson J.E.

Sponsor: N/A

Test Material: Dinoseb obtained from Dow Chemical Co., Midland, Mich., Lot # 7201203, 1968 and [$^{14}$C]dinoseb (uniformly ring labeled, 3.04 mCi/mmol; Mallinckrodt Chemical, St. Louis,Mo)

Dose Levels: 0, 14.1 and 15.3 mg/kg/day for treatment on days 10-12 of gestation; 17.7 mg/kg/day on day 11 of gestation; 18.8 mg/kg/day on day 12 gestation, half of mice pretreated with phenobarbital (2x for 3 days); 15.3 or 17.7 mg/kg/day on day 12 gestation (1 hr after pretreatment with SKF-525A)

Species: Female mice, Swiss-Webster (Spartan Research Animals, Haslett, Michigan)

METHODS:

Mice were maintained on a 12-hr light-dark cycle and received food and water ad lib. Pregnant mice were obtained by pen breeding, the day a vaginal plug was observed being designated as day 1 of gestation. Dinoseb (cold or labeled) was dissolved in dilute NaOH and adjusted to physiological pH with HCl, while phenobarbital(Mallinckrodt Chemical) and SKF-525A (Smith,Kline and French, Phil.,Pa.) were dissolved in distilled water. All agents were administered intraperitoneally in a volume of 10 ml/kg body weight.

For food deprivation studies, three groups of pregnant mice were deprived of food for 0, 24 or 48 hr beginning the 9th day of gestation; subgroups of these mice were treated with dinoseb on days 10-12 of gestation. In a second experiment 2 groups of pregnant mice were given single injections of dinoseb on day 11 or day 12 of gestation. For approximately half the mice in each group, dinoseb treatment was preceded by 50 mg/kg phenobarbital pretreatment twice daily for 3 days; the rest of the mice were not pretreated. Two other groups received dinoseb on day 12 of gestation 1 hr after treatment with 32.0 mg/kg SKF-525A. A third group was untreated.

Fetuses were removed on day 19 of gestation by Caesarian section, weighed, and examined for external anomalies. Half the fetuses of each litter were fixed in Bouin's solution and later examined for soft tissue anomalies by the method of Wilson(1965). The remaining fetuses were fixed in 95% ethanol and after clearing with 1% KOH, the fetal skeletons were stained with alizarin red S and examined with a dissecting microscope.

Pharmacokinetic studies were performed with radiolabeled material but will be discussed under the metabolism study reviews.

Statistical analyses were by the analysis of variance using between-group comparisons by the least significance difference test (Steel and Torrie, 1960). The litter was used as the unit of analysis.

Comments: This study was not intended to be submitted for regulatory requirements but as a screen for testing hypotheses concerning the factors involved in the production of teratogenic effects from ip injection of dinoseb.

RESULTS:

As presented in Table 1, food deprivation at 48 hrs alone significantly increased the number of soft tissue defects in the controls ($p<0.05$; both in relationship to the control, nondeprived and the dinoseb, non-deprived groups). At 15.8 mg/kg/day, there was a statistically significant increase in externally-observed defects ($p<.05$) compared to non-deprived controls (25%) which was significantly magnified after 24 hrs food deprivation (34% of non-deprived dinoseb group; $p<.05$) but not at 48 hrs. These anomalies were reported typically as amelia, micromelia, ectrodactyly, or brachydactyly, club foot, and ecaudia or microcaudia. In the 15.8 mg/kg/day group there was an increase in soft tissue defects compared to similarly treated controls which was statistically significant at 24 hours (40%;$p<0.05$) but not at 48 hours. Hydronephrosis was reported as the most frequent soft tissue anomaly. Fetal body weights at both 14.1 and 15.8 mg/kg/day after 24 and 48 hrs food deprivation were significantly decreased ($p<0.05$) as compared to similarly-deprived controls.

As presented in Table 2, examination of the skeletal anomalies in the offspring indicates, that as with the soft tissue anomalies, an increase related to food deprivation alone (absent or non-ossified phalanges(EL)/>200%, absent or non-ossified hallux /262%:statis. significant at $p<0.05$, fused ribs, incomplete vertebrae, split sternebrae) occurred at 48 hrs. There were a number of anomalies (statistically significant) produced by dinoseb as compared to the control (both non-deprived) at both doses but predominantly at the higher dose of 15.8 mg/kg/day including: absent or small ischium or pubis, absent or small femur, absent or small tibia or fibula, absent or nonossified hallux, fused ribs, incomplete or fused or split vertebrae, absent or nonossified or split sternebrae. For the lower dosage, 24 hr or 48 hr food deprivation appears to increase the % anomalies as compared to similar food-deprived controls roughly equally(except for the digital bones/48 hr), i.e., for the hallux, fused vertebrae and absent or non-ossified sternebrae. However at the higher more teratogenic/fetotoxic dose of 15.8 mg/kg/day, 24 hr food deprivation enhanced the effects of dinoseb administration in a statistically significant manner for a number of anomalies including the femur, tibia or fibula, digital bones, phalanges, ribs and sternebrae (absent or non-ossified). Further food deprivation up to 48 hrs resulted in no essential enhancement of the dinoseb-related anomalies.

Pretreatment of pregnant mice with phenobarbital(phb) to stimulate microsonal enzyme activity resulted in decreased % resorptions at 13.8 mg/kg/day from dinoseb administration at day 12 of gestation (5.7%/contr., 55%/dsb*vs 8% dsb-phb$^{\dagger}$), return to normal fetal body weight (1.38 g/contr., 0.79 g/dsb* vs 1.34 g/dsb-phb$^{\dagger}$) and increase in the % normal soft tissues (100%/contr., 22.2%*/dsb vs 76.7%*$^{\dagger}$/dsb+phb). However at the lower dose of 17.7mg/kg/day dinoseb administered on day 11 of gestation, phenobarbital pretreatment resulted in no discernable difference in effect from dinoseb alone except for an enhancement of the % abnormal soft tissue anomalies (100%/contr., 79.5%*/dsb vs 44.2*$^{\dagger}$/dsb+phb). SKF-525A pretreatment to inhibit drug-metabolizing enzymes plus dinoseb administration at day 12 gestation (15.8 mg/kg/day) resulted in a statistically significant increase ($p<0.05$) in % fetal resorption (5.7%/contr. vs 36%/ dsb+pnb) and in soft tissue anomalies (100%/cont. vs 29.2%/dsb+phb). The authors noted that neither of the dosages of phenobarbital or SKF-525A administered were teratogenic, or produced maternal mortality or increased resorption rates (Gibson and Becker, Teratol.1: 393398,1968). The results of SKF-525A pretreatment with administration of 17.7 mg/ kg/day were not analyzed due to the small number of litters reported surviving.

Pharmacokinetic studies with labeled material were performed in vivo and will be discussed more completely under the metabolism section. The authors reported that disposition of [$^{14}$C]dinoseb was slowed by food deprivation at 24 hrs and enhanced by phenobarbital pretreatments. Food deprivation for 48 hrs and SKF-525A pretreatment did not affect disappearance of dinoseb from plasma, but they were reported to increase and decrease the rate of disappearance from the liver, respectively.

(*significantly different from no pretreatment, no dinoseb: $p<0.05$;$^{\dagger}$ significantly different from no pretreatment that received an equivalent dose of dinoseb: $p<0.05$)

Table 1: Fetotoxicity with dinoseb treatment (days 10-12 gestation) after food deprivation (gestational days 9 or 9 and 10)

| dose (mg/kg/day) | depri-vation (hr) | # of pregn. mice[a] | resorbed or dead(%)[b] | fetal body wt.(g)[b] | external exm. (% normal)[b] | soft tissue exam(% normal)[b] |
|---|---|---|---|---|---|---|
| 0 | 0 | 14 | 6.3(2.2) | 1.39(.03) | 98.7(.9) | 83.4(3.9) |
| 0 | 24 | 14 | 11.8(5.7) | 1.36(.02) | 95.5(2.0) | 77.7(7.2) |
| 0 | 48 | 7 | 9.1(3.0) | 1.33(.02) | 96.8(2.0) | 53.5(8.6)†[c] |
| 14.1 | 0 | 7 | 4.4(2.2) | 1.27(.03) | 95.8(2.2) | 83.2(3.0) |
| 14.1 | 24 | 6 | 6.7(2.7) | 1.24(.05)*[d] | 81.6(14.9) | 62.1(15.0) |
| 14.1 | 48 | 7 | 26.9(13.7) | 1.14(.05)*† | 78.7(16.1) | 55.1(24.3) |
| 15.8 | 0 | 15 | 8.9(2.0) | 1.19(.03) | 73.7(8.0)* | 61.2(7.4) |
| 15.8 | 24 | 14 | 13.4(3.5) | 1.16(.03)* | 48.3(10.0)*† | 46.1(9.4)* |
| 15.8 | 48 | 7 | 20.4(13.5) | 1.16(.04)* | 71.3(8.1)* | 45.7(12.9) |

[a]all pregnant mice survived; [b]values are mean response/litter(SE); [c]values marked with a (†) differ significantly from those of the nondeprived groups that were treated with the same dose of dinoseb ($p<.05$); [d]values marked with an asterisk (*) differ significantly from those of the similarly deprived, no drug group ($p<0.05$)

DISCUSSION/RECOMMENDATIONS:

Food deprivation alone for 48 hrs significantly increased the number of soft tissue defects in the controls, while dinoseb treatment (15.8 mg/kg/day) alone produced major teratogenic externally observed defects compared to non-deprived controls (25%) which were significantly magnified after 24 hrs food deprivation (34% of non-deprived dinoseb group; $p<.05$) but not at 48 hrs. These anomalies were reported typically as amelia, micromelia, ectrodactyly, or brachydactyly, club foot, and acaudia or microcaudia. In the 15 mg/kg/day group there was a similar increase in soft tissue defects compared to similarly treated controls which was statistically significant at 24 hours (40%;$p<0.05$) but not at 48 hours. Hydronephrosis was reported as the most frequent soft tissue anomaly.

Examination of the skeletal anomalies in the offspring indicates, as with the soft tissue anomalies, an increase related to food deprivation alone (absent or non-ossified phalanges(HL)/>20)%, absent or non-ossified hallux /262%: statis. significant at $p<0.05$, fused ribs, incomplete vertebrae, split sternebrae) occurred at 48 hrs. There were a number of anomalies (statistically significant) produced by dinoseb as compared to the control (both non-deprived) at both doses but predominantly at the higher dose of 15.8 mg/kg/day including: absent or small ischium or pubis, absent or small femur, absent or small tibia or fibula, absent or nonossified hallux, fuse ribs, incomplete or fused or split vertebrae, absent or nonossified or split sternebrae. At the higher more teratogenic/fetotoxic dose of 15.8 mg/kg/day, 14 hr food deprivation enhanced the effects of dinoseb administration in a statistically significant manner for a number of anomalies including the femur, tibia or fibula, digital bones, phalanges, ribs and sternebrae (absent or non-ossified). Further food deprivation up to 48 hrs resulted in no essential enhancement of the dinoseb-related anomalies.

Table 1: % incidence of skeletal anomalies (gestational days 10-12) after food deprivation (gestational days 9 or 9 and 10)

| | DOSE(mg/kg/day) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | | | 14.1 | | | 15.8 | | |
| Type of anomaly | deprivation(hr) | | | deprivation(hr) | | | deprivation(hr) | | |
| | 0(14)[a] | 24(14) | 48(7) | 0(7) | 24(6) | 48(6) | 0(15) | 24(14) | 48(6) |
| digital bones(FL) abs or NO | 0[b] | 1.2 (1.2) | 0 | 0 | 5.6 (5.6) | 20.0 (16.3)*† | 6.5 (3.1) | 18.4 (8.0)* | 6.9 (3.1) |
| scibium or tibia:abs/ mall | 0 (2.0) | 1.0 | 0 (2.0) | 2.0 (11.1) | 11.1 (8.3) | 8.3 (9.0)* | 27.6 (7.1)* | 27.6 (5.3) | 14.0 |
| emur:abs r small | 1.4 (1.4) | 0 | 0 | 2.0 (2.0) | 18.0 (13.7) | 12.5 (12.5) | 19.5 (7.3)* | 40.2 (9.8)*† | 14.0 (5.1) |
| ibia/fib- la:abs/sm. | 0 | 0 | 0 | 0 | 18.0 (13.7) | 12.5 (12.5) | 20.7 (8.2)* | 46.2 (10.1)*† | 16.4 (5.9) |
| igital bones(FL): bs/NO | 0 | 1.2 (1.2) | 0 | 0 | 12.5 (8.5) | 15.8 (12.3) | 11.4 (5.4) | 40.1 (9.4)*† | 9.5 (6.0) |
| halanges FL):abs/NO | 18.5 (7.1) | 21.3 (7.5) | 40.1 (13.0) | 37.2 (12.9) | 43.2 (18.3) | 74.4 (14.0)† | 32.3 (6.9) | 58.9 (9.9)*† | 54.8 (14.4) |
| ibula:abs r NO | 27.0 (8.7) | 38.4 (5.3) | 70.8 (11.4)*† | 84.9 (8.9)* | 77.9 (11.4)* | 97.6 (2.4) | 79.8 (5.6)* | 80.2 (8.0)* | 91.7 (8.3) |
| ibs:fused | 0 | 8.3 (5.7) | 12.1 (11.2) | 3.2 (5.3) | 5.2 (3.3) | 14.0 (8.0) | 17.6 (5.6)* | 35.7 (7.4)*† | 14.3 (9.6) |
| ertebrae: ncomplete | 2.4 (2.4) | 2.1 (.4) | 16.3 (14.1) | 10.8 (4.3) | 11.1 (11.1) | 29.8 (15.0) | 26.3 (6.8)* | 37.0 (8.9)* | 12.3 (6.8) |
| ertebrae: sed | 0 | 3.6 (3.6) | 6.1 (6.1) | 23.0 (12.0) | 33.1 (15.0)* | 39.4 (14.6)* | 59.6 (9.3)* | 63.9 (9.9)* | 61.4 (10.3)* |
| ertebrae: lit | 0 | 5.7 (3.7) | 0 | 0 | 2.4 (2.4) | 0 | 17.0 (4.0)* | 14.3 (4.7) | 11.0 (7.1) |
| ernebrae: s or NO | 0 | 1.0 (1.4) | 0 | 0 | 26.4 (14.0)*† | 10.7 (8.2) | 16.0 (4.1)* | 41.0 (4.8)*† | 13.1 (7.9) |
| ernebrae: lit | 5.0 (2.9) | 20.5 (6.8) | 16.2 (8.1) | 21.3 (8.2) | 2.4 (2.4) | 30.2 (8.2) | 30.6 (5.6)* | 23.7 (4.8) | 39.8 (7.8) |

[a]From: Limb: FL=limb limb:NO=not ossified; [b]# litters examined ; =values are percent incidence mean response litter SE ; *significantly different from values of the similarly deprived, untreated group ($p<0.05$); † significantly different from values of the nondeprived group treated with the same dose of Dinoseb($p<0.05$)

Pretreatment with phenobarbital inhibited the terotogenic/fetotoxic effects dinoseb (as evidenced by a decrease in the % fetal resorptions, return to rmal fetal weights, and increase in the % normal soft tissues) at the higher dose 13.8 mg/kg/day (day 12 of gestation) but not at a dose of 17.7 mg/kg/day (day gestation). Inhibition of microsomal metabolism with SKF-525A appeared to hance the teratogenic/fetotoxic effects of dinoseb (as evidenced by a statis- cally significant increase in % fetal resorption and soft tissue anomalies with .8 mg/kg/day at day 12 gestation).

It is concluded that the teratogenic/fetotoxic effects of dinoseb administerd p. appear to be modified (enhanced or decreased) by food deprivation for 24 urs but not for 48 hours. Food deprivation of [illegible] may result in teratogenic/ totoxic effects. The mechanism of this effect may relate to in vivo metabolism nce the toxic effects of dinoseb could be alleviated by pretreatment with enobarbital which stimulates dinoseb's elimination from the body or enhanced by hibition of liver microsomal enzymes with SKF-525A pretreatment. Pharmacokinetic ta appear to support this suggestion.

This study was not intended to satisfy regulatory requirements and is desig- ted Core Supplementary.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

005421

EPA: 63-02-4225
DYNAMAC No. 1-051-81
April 8, 1985

DATA EVALUATION RECORD

DINOSEB

Mutagenicity--Multiple Genetic Toxicology Studies

STUDY IDENTIFICATION: Mutagenicity--Multiple genetic toxicology studies.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I Cecil Felkner

Date: 4-8-85

1. CHEMICAL: Dinoseb.

2. TEST MATERIAL: N/A.

3. STUDY/ACTION TYPE: Mutagenicity--multiple genetic toxicology studies.

4. STUDY IDENTIFICATION: N/A.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 4-7-86

Brenda T. Worthy, M.T.
Independent Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 4-7-86

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 4-8-86

James Rowe, Ph.D.
EPA Reviewer

Signature: James Rowe
Date: 4/18/86

Larry Ottlik, D.A.B.T.
EPA Section Head

Signature: _____
Date: _____

I. INTRODUCTION:

The purpose of this document is to summarize the review findings of 20 *in vitro* and two *in vivo* published genetic toxicology assays conducted with dinoseb. The studies are presented in tabular form and are discussed in this abbreviated data evaluation record (DER).

II. SUMMARY:

The published results of 13 gene mutation (11 *in vitro* and 2 *in vivo*) and 9 DNA repair assays with dinoseb were submitted for review. No assays investigating the clastogenic potential of the test material were presented. Relevant parameters and review findings for studies related to genetic toxicology are shown in Table 1. Listed in Table 2 are three published studies that are not related to genetic toxicology and are therefore not evaluated in this DER.

A. Gene Mutation in Somatic Cells

1. Procaryotes: Under contract to EPA, Simmon et al.(study No. 1, Table 1) evaluated S9-activated and nonactivated dinoseb (1-1000 µg/plate) with Salmonella typhimurium TA100, TA1535, TA1537, and TA1538 and Escherichia coli WP2 in plate incorporation assays. Dinoseb at 1000 µg/plate was cytotoxic for all strains with and without activation. A reduction in revertant colonies was also seen for strain TA1535 at 500 µg/plate (-S9) and TA1538 at 500 µg/plate (-S9). Slight reductions in E. coli revertant colonies were noted at S9 activated and nonactivated 500 µg/plate dinoseb. No increase in either histidine or tryptophan reversion was observed for any strain either with or without S9 activation. Although S. typhimurium TA1537 was not evaluated with either a nonactivated or S9-activated positive control, the study is considered acceptable because dinoseb was assayed up to a cytotoxic dose.

Shirasu et al. (study No. 2, Table 1) conducted an S. typhimurium and E. coli mammalian microsome mutagenicity assay with unreported doses of dinoseb. Dinoseb was considered negative as suggested by the authors' omission of dinoseb from the list of active chemicals; however, the information was insufficient to draw meaningful conclusions and hence unacceptable.

In S. typhimurium/mammalian microsome plate incorporation assay, Eisenbeis et al. (study No. 3, Table 1) found that concentrated test material, the commercially used concentration (which was not reported), and dinoseb in combination with other herbicides (concentrations not reported) were negative, both with and without S9 activation. Although no primary data were reported for the experimental agents, the authors stated, "the number of revertant colonies per plate

was never significantly different from the background spontaneous revertant rate per plate." Both the reported values for all S9 activated and nonactivated positive controls (>2000 revertants per plate) and the average number of spontaneous revertants fell within expected limits. We, therefore, concluded that the study was acceptable and that dinoseb was negative.

Similarly, there was no increase in histidine revertants of S. typhimurium TA100, TA98, TA1535, TA1537, and TA1538 or tryptophan revertants of E. coli WP2 after exposure to 50, 500 and 5000 µg/plate dinoseb with or without S9 activation (Moriya et al.; study No. 4, Table 1). The authors listed no primary data for negative compounds and did not include the conventional positive control series for this assay. We assess, however that the study was acceptable because dinoseb was tested up to an adequate dose (5000 µg/plate) and the ability of the tester strains to detect either a direct-acting mutagen or a promutagen was demonstrated by the positive response observed with 50 of the total 228 pesticides evaluated in this study.

The two remaining microbial gene mutation studies were reported as negative, but were considered unacceptable because one was conducted without S9 activation in a spot test (Andersen et al.; study No. 5, Table 1) and the other, which employed a semiquantitative concentration gradient technique to evaluate 2,4-dinitrophenol, an analogue of dinoseb (Probst et al.; study No. 6, Table 1), was not intended to be more than a preliminary compound screening survey.

2. Fungi: Gilberg (study No. 7, Table 1) failed to isolate dinoseb-resistant Rhizobium mutants following exposure of auxotrophic fungal strains to various concentrations of dinoseb. The study, however, provided no meaningful data for this evaluation and is judged unacceptable.

3. Insects: Valencia (cited in study No. 1d, Table 1) under contract to EPA investigated 0.5 and 1-4 ppm dinoseb in a sex-linked recessive lethal Drosophila melanogaster assay. The author reported that dinoseb may not have been adequately tested because of severe toxicity and the "very low concentrations" that were evaluated. Therefore, this study is considered unacceptable.

4. Conclusions for Gene Mutation: Dinoseb was investigated in bacterial gene mutation assays; the results show that dinoseb is cytotoxic but not mutagenic in bacteria. No conclusions can be drawn from studies investigating the mutagenic effect of dinoseb on fungi or insects. No in vitro mammalian cell assays were submitted for evaluation.

11.

B. Primary DNA Damage

1. Procaryotes: In a properly controlled study, one milligram/disc of nonactivated dinoseb caused preferential inhibition of DNA repair-deficient E. coli p3478 and Bacillus subtilis M45 (Simmon et al.; study No. 1, Table 1). The study was not conducted in the presence of S9 activation. The findings of these two studies, acceptable, without metabolic activation, indicate that dinoseb has the potential to interact with and cause primary DNA damage in bacteria.

At a later date but under the same EPA contract, dinoseb was evaluated in the S. typhimurium SL4525 ($rec^+$)/SL4700 ($rec^-$) differential toxicity assay (Waters et al.; study No. 1e, Table 1). The preferential inhibition of S. typhimurium SL4700 confirmed the primary DNA damage induced by dinoseb in E. coli and B. subtilis. The summarized results published by Waters et al., however, did not provide the dose at which dinoseb was active; therefore, the qualitative results could not be used in this evaluation.

The prophage induction test with nonactivated $10^{-2}$ to $10^{-9}$ M dinoseb provided no meaningful data for this review (Toure' and Stenz; study No. 8, Table 1) and thus is unacceptable.

2. Eucaryotes: Simmon et al. (study No. 1, Table 1) under contract to EPA examined dinoseb in two independent Saccharomyces cerevisiae D3 mitotic recombination assays. Genotoxicity was assessed in stationary phase cultures exposed for 4 hours to nonactivated and S9-activated doses of the test material.

In the first experiment (0.2 and 0.3% dinoseb, ±S9), 0.3% was markedly cytotoxic in the absence of S9 activation; cytotoxicity was apparent, but was diminished under S9-activated conditions. A sixfold increase in both the absolute number of mitotic recombinants/mL and the relative number of mitotic recombinants/$10^5$ survivors was reported for the 0.2% nonactivated dose; these increases occurred at 98% cell survival. S9-activated 0.2% dinoseb was negative. In the second experiment, 0.1 and 0.2% dinoseb were investigated. Under nonactivated and S9-activated conditions and at high survival (75%, -S9; 83%, +S9), ≥ threefold increases in both the absolute and relative number of mitotic recombinants was reported for 0.2% dinoseb. No appreciable increase in recombinogenic activity was seen at the 0.1% level.

The study authors' conclusion that dinoseb was negative is not consistent with the performing laboratory's criteria for a positive response, that is, "a positive response in this assay is indicated by an increase of more than threefold in the absolute number of mitotic recombinants per milliliter as well as the relative number of mitotic recombinants per $10^5$ survivors."

We disagree with the reported conclusion for the following reasons:

a. The $\geq$ threefold increase in recombinogenic activity was reproduced at a comparable dose in two independent studies.

b. High cell survival was observed at the dose causing a positive response.

Although dose-dependent increases were not demonstrated, the results suggest weak genotoxicity. We, therefore, consider the study inconclusive but classify dinoseb as presumptively positive.

Siebert and Lemperle (study No. 10, Table 1) exposed late logarithmic phase S. cerevisiae D4 for 16 hours to 100 and 1000 ppm dinoseb in the absence of S9 activation. Dinoseb at 1000 ppm was cytotoxic; a slight but not significant increase in tryptophan convertants occurred at 100 ppm. Since the assay was essentially performed with a single nonactivated dose, the study was not considered appropriate for evaluating the test material.

By contrast to the inconclusive findings of Simmon et al. with S. cerevisiae D3, Parry (study No. 9, Table 1) demonstrated a convincing dose-related increase in adenine and histidine convertants following exposure of late logarithmic phase S. cerevisiae to an amide formulation of dinoseb (185 to 1665 ppm). At doses $\geq$ 1295 ppm dinoseb was cytotoxic. However, dose-related increases in gene conversion at high survival levels were reported at 370, 555, 740, and 925 ppm. At all active levels the increase in adenine prototrophs/$10^6$ survivors was consistently higher than the frequency of histidine prototrophs. The study is acceptable and indicates that dinoseb can react directly with genetic material causing DNA damage in yeast. We caution, however, that the positive response was unconfirmed and occurred in a unique genotype of S. cerevisiae that has not been adequately validated for this type of assay.

3. Mammalian Cells: To complete the battery of assays required for EPA contract No. 68-01-2458, Simmon et al. (study No. 1, Table 1) exposed human diploid fibroblast WI-38 cells to $10^{-4}$ through $10^{-7}$ M dinoseb for 3 hours in the absence of S9 activation and to $10^{-3}$ through $10^{-5}$ M dinoseb (1-hour treatment) in the presence of S9 activation. The authors reported a suggestion of precipitation at $10^{-4}$ without S9 activation, no precipitation was reported under S9 activated conditions. The liquid scintillation counting of unscheduled DNA synthesis (UDS) indicated that dinoseb was not genotoxic. No cytotoxic response was shown at any assayed level, however, as stated by the authors the highest noncytotoxic dose was evaluated. Hence we concluded that dinoseb was adequately tested and the study is acceptable.

   In another study a single noncytotoxic dose (50 nmol/mL), 2,4-dinitrophenol showed no net increase in nuclear grain counts in the UDS assay using primary rat hepatocytes (Probst et al.; study No. 11, Table 1). This study, however, provided no relevant data because the entire dinoseb molecule was not investigated.

4. Genotoxic Effects in Germinal Cells: The findings of the mouse sperm morphology study, in which male mice received five daily gavage administrations of 2, 4.3, 9.3, 20, and 43 mg/kg dinoseb, provided no meaningful data (Osterloh et al.; study No. 12, Table 1). The authors concluded that dinoseb may not have been administered by an appropriate route and the selected sampling interval may have been insufficient to detect mutagenic effects on early and/or late stages in the cell cycle. Hence this study is considered unacceptable.

5. Conclusions for Primary DNA Damage: A review of studies in this category shows that dinoseb causes primary DNA damage in bacteria, is presumptively genotoxic in yeast, and is not reactive in human fibroblasts.

## III. OVERALL CONCLUSIONS:

Dinoseb was shown to induce primary DNA damage in procaryotes. Based on the inconclusive evidence of marginal recombinogenic activity in S. cerevisiae D3 and a convincing dose-related positive response in an unusual strain of S. cerevisiae, dinoseb is considered presumptively genotoxic in yeast. However, the lack of a genotoxic response in mammalian cells, which have greater cellular organization and more efficient DNA-repair capabilities than yeast or bacteria, tends to diminish the importance to humans of dinoseb-initiated DNA damage in procaryotes or lower eucaryotes. It is noteworthy that although dinoseb exhibited DNA-damage activity in E. coli, B. subtilis, and S. typhimurium, it did not cause gene mutations in the same enteric microorganisms.

We assess that the potential of dinoseb to induce gene mutation in mammalian cells was not adequately tested by the studies submitted. Similarly, the clastogenic potential of the test material has not been investigated.

An overall genetic toxicology profile for dinoseb cannot be established for the reviewed studies. It is recommenced, therefore, that the following assays be performed:

1. Gene mutation in mammalian cells (i.e., mouse lymphoma, Chinese hamster ovary cell/HGPRT, or V79 Chinese hamster cells).

2. Chromosomal aberrations (i.e., in vitro mammalian cells and/or in vivo rodent bone marrow or micronucleus assays).

TABLE 1. SUMMARY OF MUTAGENICITY TESTING OF DINOSEB

| Study/Authors/Study Identification | Genetic Endpoint | Indicator Organism | Purity | Application | Dose Range | Response | Conclusion (Evaluation) | Comment |
|---|---|---|---|---|---|---|---|---|
| Evaluation of Selected Pesticides as Chemical Mutagens: In vitro and in vivo Studies/Simmon V. F., Mitchell, A. D., Jorgensen, T. A./ Standford Research Laboratory: Report No. EPA-600/1-77-028, contract No. 68-01-2458; ORTD 132582, 5009139, and 24901 (Technical Report/Environmental Health Effects Series Report) dated 1977 | Gene mutation | S. typhi murium strains TA100, TA153, TA1537, and TA1538 | 97.1% | Plate incorporation assay | 7 doses (1 to 1000 µg/plate, ±S9 | Negative cytotoxic strains at 1000 µg/plate, ±S9 for all | Acceptable | A positive control was not used with TA1537, ±S9; however dinoseb was assayed up to a cytotoxic dose |
| | Gene mutation | E. coli WP2 | 97.1% | Plate incorporation assay | 6 doses (1 to 1000 µg/plate), ±S9 | Negative; cytotoxic at 1000 µg/plate, ? | Acceptable | Assayed up to a cytotoxic dose in a properly controlled study |
| | DNA damage | E. coli W3110 and p3478; B. subtilis H17 and M45 | 97.1% | Spot test | 1 mg/disc, -S9 only | Preferential inhibition of both repair-deficient strains | Acceptable | Acceptable for nonactivation only |
| | DNA damage (mitotic recombination) | S. cerevisiae D3 | 97.1% | Stationary phase cells exposed in suspension for 4 hours at 30°C | Study No. 1: 0.2 and 0.3% dinoseb, ±S9 | Cytotoxic ±S9 at 0.3%; sixfold increase in the absolute and relative number of mitotic recombinants at 0.2%, -S9, with high survival (90%) | Inconclusive; presumptive positive | In conflict with performing laboratory's criteria for a positive response; dinoseb was reported as negative |

(Continued)

TABLE 1. SUMMARY OF MUTAGENICITY TESTING OF DINOSEB (Continued)

| Study/Authors/Study Identification | Genetic Endpoint | Indicator Organism | Purity | Application | Dose Range | Response | Conclusion (Evaluation) | Comment |
|---|---|---|---|---|---|---|---|---|
| | | | | | Study No. 2: 0.1 and 0.2% dinoseb, ±S9 | >threefold increase in the absolute and relative number of mitotic recombinants at 0.2%, ±S9, with high survival (7%, -S9, 0%, +S9); 0.1% was negative | Inconclusive; presumptive positive | In conflict with performing laboratory's criteria for a positive response; dinoseb was reported as negative |
| | DNA damage | Human diploid fibroblasts WI-38 cells | 97.1% | Cells exposed for 3 hours without S9 or 1 hour with S9 activation | $10^{-4}$ to $10^{-7}$ M, -S9 | Negative | Acceptable (compound precipitation at $10^{-4}$ M) | Unscheduled DNA synthesis measured by liquid scintillation counting procedures |
| | | | | | $10^{-3}$ to $10^{-5}$ M, ±S9 | Negative | Acceptable (no compound precipitation, assayed at highest non-cytotoxic dose) | |

a In vitro and in vivo studies of selected pesticides to evaluate their potential as chemical mutagens/Newell, G. W./Toxicological Methods and Genetic Effects Workshop: Proc. of Symposium Vol. 2: 141 150 (July 30 Aug. 1, 1975)

Published progress report of results summarized above for EPA contract 68-02-2450 (1977).



(Continued)

| Study/Authors/Study Identification | Genetic Endpoint | Indicator Organism | Purity | Application | Dose Range | Response | Conclusion (Evaluation) | Comment |
|---|---|---|---|---|---|---|---|---|
| Unscheduled DNA synthesis testing of substitute pesticides/Mitchell, A. D./Toxicological Methods and Genetic Effects Workshop: Proc. of Symposium, Vol. 2: 151-153 (July 30-Aug. 1, 1975) | Published progress report of results summarized above for EPA contract 68-01-2450 (1977). | | | | | | | |
| Pesticides: Mutagenic and carcinogenic potential/Waters, M. D. Nesnow, S., Simmon, V. F., Mitchell, A. D., Jorgenson, T.A., Valencia, R./ACS Symposium 160: 80 113 (1981). | Published report of results summarized above for EPA contracts 68-01-2450 and 68-01-2474 (1977). | | | | | | | |
| An overview of short term tests for the mutagenic and carcinogenic potential of pesticides/Waters, M. D., Simmon, V. F., Mitchell, A. D., Jorgenson, T. A., Valencia, R./J. Environ. Sci. Health B15(6): 859-966 (1980) | Published report of the evaluation of selected pesticides as chemical mutagens in vitro and in vivo studies, EPA 68011-77-020, contract no. 68-01-2450, which is summarized above. In addition, this report contained the results of pesticides evaluated in D. melanogaster (EPA contract 68-01-2474, 1977 author; Valencia, R.) | | | | | | | |
| | Gene mutation in insects | Pre- and postmeiotic male germinal cells | Technical grade | $P_1$ males allowed to feed on test material for 24 hours | 0.5 and 1-4 ppm | Negative | Unacceptable; due to excessive toxicity and the low dose(s) evaluated the dose range may have been inadequate as reported by the author. | Compound not adequately tested |
| Study of pesticide genotoxicity/ Waters, M. D., Sandhu, S. S., Simmon, V. F., Mortelmans, K. E., Mitchell, A. D., Jorgenson, T. A., Jones, D. C. L., Valencia, R., Garrett, N. E./1. Basic Life Sci. 21: 275-336 (1982) | Published report of results summarized above for EPA contract 68-01-2450 and 68-01-2474 (1977). In addition, this report contained the qualitative results of the S. typhimurium SI4525 ($rec^+$)/SI4700 ($rec^-$) differential toxicity assay with dinoseb. | | | | | | | |

(Continued)

TABLE 1. SUMMARY OF MUTAGENICITY TESTING OF DINOSEB (Continued)

| Study/Authors/Study Identification | Genetic Endpoint | Indicator Organism | Purity | Application | Dose Range | Response | Conclusion (Evaluation) | Comment |
|---|---|---|---|---|---|---|---|---|
| | DNA damage | S. typhimurium SI4525 (rec$^{+}$)/ SI4700(rec$^{-}$) | Technical grade | Spot test | Not reported | Positive | Not applicable summary data | No information on dose |
| 2 Mutagenicity screening studies on pesticides/Shirasu, Y., Moriya, M., Iezuka, H., Teramoto, S., Ohta, T., Inoue, T./Environ. Mutagen. Carcinogen. Proc. Intl. Conf. 3: 341-345 (1982) | Gene mutation | S. typhimurium TA1535, TA1537, TA98, TA100 and E. coli WP2 | Not reported | Not reported | Not reported; tested t59 | Negative by omission from chemicals reported active | Unacceptable | |
| 3 The Ames mutagen assay tested against herbicides and herbicide combinations/Eisenbeis, S. J., Lynch, D. L., Hampel, A. L./Soil Sci. 133(1): 44-47,(1982) | Gene mutation | S. typhimurium TA1535, TA1537, TA1538, TA98, and TA100 | Purity not reported; dinoseb; dinoseb naptalam; dinoseb lasso | Plate incorporation assay | Concentrated solution, commercially used concentration, and in combination with other herbicides, t59 | Negative | Acceptable | Concentration test material assayed in a properly controlled study |
| 4 Further mutagenicity studies on pesticides in bacterial reversion assay systems/Moriya, M., Ohta, T., Watanabe, K., Miyazawa, T., Kato, K., Shirasu, Y. Mutat. Res. 116: 185-216 (1983) | Gene mutation | S. typhimurium TA100, TA98, TA1535, and TA1538<br><br>E. coli strain WP2 | Not reported | Plate incorporation assay | 0, 50, 500, 5000 µg/plate, t59 | Negative | Acceptable | Maximum recommended dose assayed; no positive controls; however, 50 of 220 pesticides evaluated were positive, hence sensitivity of the test system was demonstrated |

(Continued)

TABLE 1. SUMMARY OF MUTAGENICITY TESTING OF DINOSEB (Continued)

| Study/Authors/Study Identification | Genetic Endpoint | Indicator Organism | Purity | Application | Dose Range | Response | Conclusion (Evaluation) | Comment |
|---|---|---|---|---|---|---|---|---|
| 5 Evaluation of herbicides for possible mutagenic properties/ Andersen, K. J., Leighty, E. G., Takahashi, M. T./J. Agric. Food Chem. 20(3): 649 656 (1972). | Gene mutation | S. typhi murium, 8 histidine deficient strains | Not reported | Spot test (-S9 only) | 1-5 µl spotted | Negative | Unacceptable (no test) | Protocol technically deficient (no S9 activation, qualitative method) |
| 6 Chemically induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds/Probst, G. S., McMahon, R. E., Hill, L. E., Thompson, C. Z., Epp, K. K., Neal, S. B./ Environ. Mutagen. 3: 11 32 (1981) | Gene mutation | S. typhi murium strains TA1535, TA100, C3076, TA1537, D3052, TA1538, and TA98; E. coli WP2 and WP2 uvrA | Not reported; test material: 2,4 dinitro phenol | Concentration agar gradient test | 1:10,000 fold concentration range, +S9 | Negative | Not Applicable | Semiquantitative technique; intended as screening study of a structural analogue of dinoseb |
| 7 On the effects of some pesticides on Rhizobium and isolation of pesticide resistant mutants/ Gillberg, R. G./ Arch. Microbiol. 75(1): 203 208(1971) | Gene mutation in fungi | R. meliloti, R. leguminosarum, R. trifolii | 50% | Isolation of pesticide resistant mutants | 50, 100 150 µg/ ml | Negative | Not Applicable | |
| 8 The action of selected herbicides on bacteriophages and Escherichia coli/Toure', I. M. and Stenz, E./ Zentralblatt Bakt. II Abt. 60 132: 163 177 (1977) | DNA damage | E. coli C600, W1649 with λ and M12 phages | Not reported | Prophage induction test | $10^{-2}$ to $10^{-9}$ M | Negative | Unacceptable (no test) | No S9 activation; methodology outdated |

(Continued)

| Study/Authors/Study Identification | Genetic Endpoint | Indicator Organism | Purity | Application | Dose Range | Response | Conclusion (Evaluation) | Comment |
|---|---|---|---|---|---|---|---|---|
| 9 The induction of gene conversion in yeast by herbicide preparation/ Parry, J. M./ Mutat. Res. 21: 83-91 (1973) | DNA damage (mitotic gene conversion) | S. cerevisiae diploid strain; heteroallelic at adenine and histidine loci | Amine formulation of dinoseb | late log phase cells exposed in suspension for 18 hours at 28° C, S9 only | 9 doses ranging from 105 to 1665 ppm | Positive; dose related increase in adenine and histidine convertants | Acceptable without S9 activation only | |
| 10 Genetic effects of herbicides: Induction of mitotic gene conversion in Saccharomyces cerevisiae/ Siebert, D. and Temperle, F./ Mutat. Res. 27:111-120 (1974) | DNA damage (mitotic gene conversion) | S. cerevisiae D4 | Not reported | log phase cells exposed in suspension 18 hours at 25°C ( S9 only) | 100 and 1000 ppm | Cytotoxic at 1000 ppm; negative at 100 ppm | Unacceptable | Protocol technically deficient (no S9 activation); only one noncytotoxic dose assayed |
| 11 Chemically induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds/Probst, G. S., McMahon, R. E., Hill, L. E., Thompson, C. Z., Ipp, K. K., Neal, S. B. Environ. Mutagen. 3: 11-32 (1981) | DNA damage | Primary rat hepatocytes; | Not reported, test material: 2,4 dinitrophenol | cells exposed 20 hr at 37°C | 0.5 to 1000 nmoles/mL | Negative | Not applicable | (Survey study) highest noncytotoxic dose (50 nmol/mL) was the only level counted; intended as a screening study of a structural analogue of dinoseb |
| 12 An assessment of the potential testicular toxicity of 10 pesticides using the mouse sperm morphology/Bruce, J., Teir, G., Pond, S. and Becker, C./Mutat. Res. 116:407-415 (1983) | DNA damage/ repair in germinal cells | Mouse sperm | 98% | five daily doses by gavage | 2, 4.3, 9.3, 20, 43 mg/kg/ day | Negative | Unacceptable | The authors concluded that the route of administration may have been inappropriate and the entire spermatogenesis and spermiogenesis cycles were not sampled |

(Concluded)

TABLE 2. SUMMARY OF STUDIES ON DINOSEB NOT RELATED TO GENETIC TOXICOLOGY

| udy/Authors/Study Identification | Application |
|---|---|
| the selective effects of herbi<br>ides upon the growth of yeast<br>uthors/Parry, J. M. / Arch<br>Microbiol. 98, 111 118 (1974) | Physiology |
| ytotoxic responses of selected<br>insecticides in chick ganglia<br>uthors/Sharma, R. P. and<br>Oberstelner, E. J. / Can. J.<br>omp. Med. 45, 60 69 (1981) | In vitro cytotoxicity |
| Screen for pesticide toxicity<br>o protein and RNA synthesis<br>n Hela Cells/Hyba, R. E. /<br>J. Agric. Food Chem. 21 (1)<br>62 367 (1973) | Protein synthesis |

STUDY EVALUATION

W. Totter
Secondary Reviewer

| | |
|---|---|
| Study Title: | Effects in mice of high and low environmental temperature on the maternal and fetal toxicity of 2-sec-butyl-4,6-dinitrophenol (dinoseb) and on disposition of [$^{14}$C]-dinoseb |
| Reference: | N/A |
| Testing Facility: | Department of Pharmacology, Michigan State University, East Lansing, Michigan 48824 |
| Final Report No.: | Teratology, 12:147-154 |
| Final Report Date: | October, 1975 |
| Study Authors: | Preache M.M., Gibson J.E. |
| Sponsor: | N/A |
| Test Material: | Dinoseb (Dow, Midland, Michigan, Lot # 720206, 196#) and [$^{14}$C]dinoseb (uniformly ring-labeled, 3.04 mc/mmole: Mallinkrodt, St. Louis, Missouri) |
| Dose Levels: | Test material was dissolved in 0.1 N NaOH, returned to physiological pH with 0.1 N HCl, and administered ip in a volume of 10 ml/kg body weight at 6.3 mg/kg (25 uCi/kg) or 14.1 mg/kg (25 uCi/kg) of [$^{14}$C]dinoseb |
| Species: | Mice, nonpregnant females of Swiss Webster strain (Spartan Research Animals, Haslett, Michigan) |

METHODS:

Mice were maintained on a 12 hr light-dark cycle and received food and water ad lib. Nonpregnant animals (16/group) were treated with ip injection of [$^{14}$C]dinoseb (6.3 mg/kg: 25uCi/kg) and placed in an incubator at 32°C or left in their home cage at room temperature until killed. In a second experiment 14.1 mg/kg [$^{14}$C]dinoseb (25 uCi/kg) was administered to 32 nonpregnant females. Half the mice were wet and placed in the cold room (0°C) for 2 hr followed by injection or were left dry at room temperature. In both groups barriers prevented the 2 mice in each cage from huddling during the 2 hr following injection.

Three, 6, 12, or 24 hr after compound administration of radioactive dinoseb samples of blood were obtained by cardiac puncture under ether anesthesia. Plasma was separated by centrifugation and 100 ul added to glass scintillation vials. Samples of liver, kidney, and lung were collected, minced, added to tared vials, and weighed. The samples were solubilized in 1-2ml of Soluene 100®, and after addition of 15 ml toluene counting solution (5 g PPO plus 200 mg dimethyl POPOP/l toluene), radioactivity were measured with a liquid scintillation counter and expressed as dpm/ul plasma or mg tissue.

The t test for differences between slopes (Goldstein, 1964) was used to evaluate the effect of temperature on the rate of disappearance of [$^{14}$C]dinoseb from plasma and tissues.

Comments:

This study was obtained from the open literature and was not designed to meet the requirements for a standard metabolism study.

RESULTS/DISCUSSION/RECOMMENDATIONS:

Curves for the rate of disappearance of radioactivity (dpm/ul plasma or mg tissue) in the plasma, liver, kidney and lung at 3-24 hours after administration of either 6.3 or 14.1 mg/kg (both 25 uCi/kg) were presented for animals maintained at 0°(wet), 14° and 32°C. There were no differences in the rate of disappearance for animals kept under any of these conditions. The authors had shown, in the same report, that increased embryotoxicity would occur if the temperature of the dams were elevated to 32°C. Based on their findings with the nonpregnant females, they suggested that the increased embryotoxicity was not related to an inhibition in the rate of disappearance since there were no differences in this parameter for mice kept at 24 or 31°C. They also noted that, while caution was necessary in extending the pharmacokinetic results from the nonpregnant to the pregnant situation, a previous study (Gibson and Rao, Fd. Cosmet. Tox. 11:45-52, 1973) had shown that the disappearance of dinoseb from the plasma of pregnant and nonpregnant female mice was not different.

This report is designated as Core Supplementary data.

005421

STUDY EVALUATION

W. Teaters
Secondary Reviewer

| | |
|---|---|
| Study Title: | Electron-capture gas chromatographic determination of 2-sec-butyl-4,6-dinitrophenol (DNBP) residues in feed, tissue, and excreta |
| Reference: | N/A |
| Testing Facility: | U.S. EPA, Health Effects Research Laboratory, RTP, NC 27711 |
| Final Report No.: | J. Agric. Food Chem., 26(2): 425-428 |
| Final Report Date: | 1978 |
| Study Authors: | Edgerton T.R. and Moseman R.F. |
| Sponsor: | N/A |
| Test Material: | Technical dinoseb; DNBP(dinoseb) analytical reference standard of 99% purity obtained from the EPA Pesticide Repository, RTP, NC |
| Dose Levels: | 50 ppm and 200 ppm in rat chow fed to six rats/dose for 138 days |
| Species: | Rats, Sherman strain |

METHODS:

Eighteen Sherman rats weighing about 250 g were housed in individual conventional cages having free access to water and feed. The animals were conditioned to their environment before the feeding study began. After the conditioning period, the DNBP was incorporated into the feed ration as follows: One part DNBP (technical) in acetone, dissolved in two parts peanut oil, was added to pre-mix chow and placed in a large Baker's Mixer. The concentrate chow contained 2000 ppm DNBP. The concentrate was diluted with chow to give final concentrations of 50 and 200 ppm DNBP. Six rats were continued on a DNBP-free diet while six rats were fed 50 ppm for 138 days and six rats fed 200 ppm for 138 days also. Three groups of four rats each were placed in metabolism cages for collection of urine and feces for the overnight period immediately prior to sacrifice. During this period, all rats were fed plain chow. All animals were killed after 138 days with two rats from each diet level used for tissue analysis. All tissue samples were stored in scintillation vials and frozen immediately after sacrifice. Feces were stored in specimen bottles and frozen prior to analysis.

An analytical standard of DNBP was prepared in benzene and stored at -15°C in a brown glass bottle. A solution of DNBP in benzene was methylated by adding 5 ml of diazomethane reagent in a well-ventilated hood. The methylated standard was allowed to stand for 30 minutes before EC-GC analysis.

Methylated DNBP was chromatographed on acid alumina and eluted from the column with 10 ml of 60% benzene in hexane.

Analysis of feed, tissue and excreta was as follows.

Feed: A 2 g sample of freshly fortified feed was weighed into a 100 ml round-bottom centrifuge tube and extracted with a tissue homogenizer with 25 ml of benzene for approximately 2 minutes. After extraction, the sample was centrifuged and the supernatant layer transferred through anhydrous $Na_2SO_4$ into a 100 ml volumetric flask. The extraction was repeated twice more, combining the extracts and adjusting the volume to 100 ml. An aliquot was methylated and analyzed by EC-GC. A different extraction procedure was used for feed samples which had aged over 1 day.

Fat: Approximately 500 mg of tissue was extracted with three 3 ml portions of acetonitrile in a Duall Tissue Grinder. The samples was centrifuged after each extraction and the supernatant layers were combined in a 40 ml screw-cap centrifuge tube. The extract was partitioned with two 5 ml portions of hexane and centrifuged after each extraction, and the hexane layer was discarded. Twenty-five milliters of acidic (pH 2) 2% aqueous $Na_2SO_4$ was added to the acetonitrile and subsequently extracted with two 5 ml portions of hexane. The hexane extracts were combined, concentrated to a volume of 0.3-0.5 ml under a gentle stream of nitrogen, and methylated with 5 ml of diazomethane reagent. The samples were then extracted using a Duall Tissue Grinder. The sample was centrifuged, and the supernatant layers were combined in a 40 ml screw-cap centrifuge tube. The extract was evaporated under a stream of $N_2$ just to dryness. Nine milliters of acetonitrile was added and carried through the same liquid-liquid partitioning described. Methylation was allowed to proceed overnight prior to column cleanup.

Liver: Approximately 500 mg of tissue was homogenized with 5 ml of 6 N HCl in a Duall Tissue Grinder. The homogenate was transferred to a 150 ml screw-cap cul-

ture tube, sealed, and placed in a boiling water bath for 1 hr with periodic shaking. The hydrolyzed liver homogenate was extracted twice for 1 hr each on a mechanical rotator at 30-50 rpm using two 5 ml portions of hexane. The sample was centrifuged, and the extracts were combined in a 40 ml screw-cap centrifuge tube. The hexane fraction was extracted with two 5 ml portions of acetonitrile, combining the acetonitrile fractions in a 40 ml screw-cap centrifuge tube. The sample extract was carried through the same liquid-liquid partitioning and methylation as for the brain.

Blood: One gram of oxalated whole blood was weighed into a 125 mm screw-cap culture tube and 3 ml of 6 N HCl was added. The tube was sealed and placed in a boiling water bath for 1 h with periodic shaking. The sample was extracted twice for 1 hr each on a mechanical rotator at 30-50 rpm using 5 ml portions of 1:1 (v/v) ethyl ether-hexane. The sample was centrifuged, and the combined extracts were carried through the same concentration and methylation procedure as for fat. Since DNBP levels exceeded 10 ppm, column cleanup was found to be unnecessary in this study.

Urine: Two milliliters of urine were transferred to a 125 mm screw-cap culture tube and hydrolyzed with 0.4 ml of concentrated HCl. Extraction, concentration, and methylation were performed as previously described for blood.

Feces: One gram of dried, pulverized feces was weighed into a 150 mm screw-cap culture tube. Seven milliliters of 6 N HCl was added to the tube, shaken, and allowed to stand until frothing ceased. Hydrolysis, extraction, partitioning, methylation, and column cleanup followed the previously described method for liver. Methylation was completed in 1 hr.

Comments:

This study was designed to evaluate a method for quantifying dinoseb residues in feed and rat tissues, not for determining its metabolic fate.

RESULTS/DISCUSSION/RECOMMENDATIONS:

Average recoveries from freshly fortified feed, various tissues and excreta ranged from 98% (feed) to 82 % (liver) with most averaging 85% or better. The method sensitivity was estimated at 0.1 ppm for tissue and excreta samples.

Difficulty was reported in the analysis of aged fortified feed using Polytron extraction and organic solvents. It was assumed by the authors initially that solvent extraction of feed without hydrolysis was sufficient. Feed samples from the DNBP feeding study showed losses of 18% at the 50 ppm level and 29% at the 100 ppm level 25 days after fortification.

Due to these results, a control experiment was done with and without hydrolysis. At 90 days after DNBP addition (50 ug), there was an apparent recovery of 11.5 ug (75% apparent loss) without the use of acid hydrolysis. With acid hydrolysis at 180 days after DNBP addition to the feed, there was 46.8 ug (94%) of DNBP recovered.

The stability of the analytical standard of dinoseb was examined in clear and brown glass bottles. DNBP (dilute solution), stored in the clear glass bottles,

Table: DNBP residues from the feeding study (taken from Table VI, p. 428 of report)

| sample | 50 ppm level[ab] ppm found) | 200 ppm level[ab] (ppm found) | control |
|---|---|---|---|
| adipose | 0.88 | 3.46 | <0.1 |
| brain | 0.70 | 1.16 | <0.1 |
| liver | 0.29 | 1.06 | <0.1 |
| blood | 15.4[c] | 39.7 | <0.1 |
| urine | 0.99[c] | 1.01[c] | <0.1 |
| feces | 2.07[c] | 1.73[c] | <0.1 |

[a] actual DNBP based on 80% technical was 40 and 160 ppm; [b] average two determinations, two samples; [c] average two determinations, one sample pooled urine and feces

showed a significant loss over time with a 27% loss by 72 hours of storage. No apparent loss was observed in the brown glass bottles during the same time interval. Therefore, standards of the unmethylated DNBP were kept in brown glass bottles.

Examination of tissue and excreta DNBP residues from a 138 day feeding study in rats is presented above in the table. Blood is the major tissue site for dinoseb concentration, of the tissues examined. There was a proportional increase (4x) in dinoseb residues in adipose tissue and liver with blood exhibiting a three-fold increase when the DNBP concentration in the feed was increased from 40 to 160 ppm.

In conclusion, a detailed methodology for the determination of DNBP in feed, assorted tissues, and excreta was developed for a long-term feeding study in rats (138 days). Using this method, recoveries of 85% or greater were achieved. Examination of various tissues at the end of the feeding study indicates that DNBP is primarily concentrated in the blood.

This study is designated as Core Supplementary data.

005421

STUDY EVALUATION

W. Tietze
Secondary Reviewer

Study Title: Transformation of 2-sec-butyl-4,6-dinitrophenol and its esters in the animal organism (translation from German; unpublished study received Jul 22, 1965 under 270-2019)

Reference: MRID 33636, 60766

Testing Facility: Federal Bureau of Health, Max Pettenkofer Institute, Laboratory of Nutritional Hygiene

Final Report No.: Arzneimittel-Forschung 14(2): 81-84

Final Report Date: 1964

Study Authors: Ernst W. and Baer F.

Sponsor: N/A

Test Material: 2-sec-butyl-4,6-dinitrophenol (DNBP) ; 2-sec-butyl-4,6-dinitrophenol-acetate (DNBP-acetate) ; 2-sec-butyl-4,6-dinitrophenol-1,1-dimethylacrylate (DNBP-1,1-dimethylacrylate)

Dose Levels: Single oral doses(feeding) of:
DNBP: 13.8, 30 mg/kg in rabbits; 5.3, 8.9, 11.5 mg/kg in rats
DNBP-acetate: 30 mg/kg in rabbits; 6.5, 14.5, 21.8 mg/kg in rats
DNBP-1,1-dimethylacrylate: 64.0, 80 mg/kg in rabbits; 13.2, 22.0, 28.2 mg/kg in rats

Species: Rabbits and rats (number and strain unspecified)

METHODS:

Rabbits and albino rats were obtained from the test laboratory's inbred colonies. The urine was extracted three times with a threshold volume of ether or continuously extracted (pH 3). The urine extracts were concentrated in a vacuum and, for quantitative studies, brought up to a specific volume with ethanol. Descending chromatograms were made on S and S paper 2043 b mgl in a glass tank with mobile solvents. Metabolites were identified from their reaction from spraying with 1) 1 5 NaOH: dinitro-compounds=yellow, amino-nitro compounds=orange-red, 2) 10% KCN solution (aqueous) and heating at 110°: m-dinitro compounds=red, 3) 0.5% solution of $NaNO_2$ in 1 N HCl and with an 0.5% solution of b-naphthol in 1N HCl: aromatic amines=violet.

Identification and isolation of 2-sec-butylamino-4,6-nitrophenyl-6-glucuronide and b-methyl -5-(2-hydroxy-3,5-dinitrophenyl)-propionic acid from rabbit urine

750 mg INBP-acetate was fed to rabbits and the urine extracted daily with ether at pH 3. The pooled concentrates of the extracts were separated by paper chromatography with the mobile solvent. Both substances were separated with $H_2O$, eluted, and the eluate re-extracted with ether (extracts A and B). To obtain b-methyl-b-(2-hydroxy-3,5-dinitrophenyl)-propionic acid, extract A was concentrated and developed on paper chromatography with mobile solvent 3, eluted with water, re-extracted with ether, concentrated, and, separated on glass plates coated with Kieselgel G using $CHCl_3CH_2H_5OH$ (9:1). The yellow substance spot was eluted with $CHCl_3$ and, for separation from residual hippuric acid, separated again on Kieselgel G with $CHCl_3CH_2H_5OH/(CH_3)_2NH$ (90:9.5:0.5). After extraction of the substance spots with $CHCl_3$, the solvent was "quenched" and the residue re-crystallized twice from benzene. The material crystallized was pale yellow in nature with a fusion point of 161°C and a mixed melting point with synthetic compound of 161°. The IR spectrum was compared against a reference.

Isolation of 2-sec-butylamino-4,6-nitrophenyl-o-glucuronide

Extract B was extracted with ether at pH 3. After removal of the ether, the residue was dissolved in 10 ml 5 N $H_2SO_4$ and acidified with 2 N HCl at pH 3. The precipitate thus formed was filtered off after 30 minutes, dissolved in 1.5 ml 1N NaOH, and poured into 1.5 ml 2 N HCl and filtered immediately. After adjustment of the filtrate to ca. pH 3 with 1 N NaOH, it was left for 30 minutes in the cold until it crystallized out. After filtering and drying, the almost colorless, nodular, crystallized glucuronide had a melting point of 220°. Several recrystallizations were performed and the melting point and mixed melting point were identical to the authentic standard (224°). The IR spectrum was identical also.

Alkaline hydrolysis of the glucuronide occurred readily on heating in a boiling water-bath in 0.5 N NaOH solution. The 2-sec-butylamino-4,6-nitrophenol thus formed was extracted with ether at pH 3 and compared chromatographically, and via absorption spectroscopy ($lambda_{max}$: 465 mu in 0.5 N NaOH), with authentic 2-sec-butylamino-4,6-nitrophenol, and found to be in agreement. Glucuronic acid was identified with naphthoresorcinol. The glucuronide could be recovered uncharged from acid and alkaline solutions. The authors noted that since the amino groups were present in the free form they could only be dealing with the b-glucuronide of 2-sec-butyl amino-4,6-nitrophenol.

Identification of DNBP separated from the urine was achieved with paper chromatography by absorption spectroscopic measurements (lambda$_{max}$ 375 mu, shoulder at 420 nu in 1 N NaOH), and also by paper chromatographic comparison of the 2-sec-butylamino-4,6-nitrophenol formed from DNBP by reduction with $Na_2S$ with the authentic substance. In addition, DNBP, in contrast to the other dinitro metabolites, was volatile in the presence of water vapor.

Substance IV was considered to be a dinitro compound, on the basis of its color reactions. The metabolite is practically non-volatile in water vapor; it has an absorption spectrum qualitatively identical to that of DNBP and can be extracted from an alkaline solution with methylethylketone as a yellow salt.

Reference materials were synthesized in the test laboratory and included: 2-sec-butyl-4,6-dinitrophenol, 2-sec-butylacetamino-4,6-nitrophenol, 2-sec-butyl-5,7-nitrobenzoxazol, b-methyl-b-(2-hydroxy-3,5-dinitrophenyl)-propionic acid, b-methyl-b-(2-hydroxyphenyl)-propionic acid, b-methyl-b-(2 hydroxy-3,5-dinitrophenyl)-propionic acid, 2-sec-butylamino-4,6-nitrophenyl-o-glucuronide, 2-(2-hydroxyphenyl)-butanol-2, and 3-(2-hydroxyphenyl)-butanol-1.

For the determination of DNBP and substance IV, a method described by Parker (Analyst 74:646, 1949) was used. In this method, the alkali salts of both dinitro compounds were extracted with methyl ethyl ketone and determined photometrically at 430 mu. Since the extinction coefficient for Substance IV could not be ascertained, that of DNBP was used for the calculation, and hence the values for these metabolites had to be regarded as slightly uncertain. 2-sec-Butylamino-4,6-nitrophenyl-glucuronide could be readily hydrolyzed in an alkaline solution, while heating, to orange-red 2-sec-butylamino-4,6-nitrophenol with an absorption maximum at 465 mu. b-Methyl-b-(2-hydroxy-3,5-dinitrophenyl)-propionic acid, was determined photometrically in an alkaline solution at 375 and 420 mu, respectively.

Comments:

In addition to the lack of individual data, this metabolism study examined only the urine and not the various organs and tissues requested by the 1982 EPA Testing Guidelines.

RESULTS:

The authors reported that five different compounds were identified from the urine of rabbits after a single feeding (20 mg/kg dinoseb). Similar substances were identified after a single feeding in the rabbits of DNBP-acetate (60 mg/kg) and DNBP-1,1-dimethylacrylate (60 mg/kg). The five substances (substances I-V) were identified as dinoseb or dinoseb liberated from its ester(I), 2-sec-butylamino-4,6-nitrophenol(II), 2-sec-butylamino-4,6-nitrophenol-o-glucuronide (III), and b-methyl-2(2-hydroxy 3,5-dinitrophenyl)-propionic acid (V). Substance IV was not identified.

After feeding rats with DNBP and the acetate or dimethylacrylate, Substances I, IV, and V could be demonstrated in the urine but only traces of Substance II (if any) and the o-glucuronide were not excreted. If the rats were fed 2-sec-butyl-4,6-dinitrophenol, some of it was converted into the same glucuronide (Substance III) as in the rabbit.

DISCUSSION/RECOMMENDATIONS:

Free DNBP (Substance I), its acetic acid ($-COCH_3$) and 1,1-dimethylacrylic acid ester ($-CO-CH-C[CH_3]_2$) are transformed in the same way in rats and rabbits (side chain oxidation and 1 $NO_2$ group reduction) with hydrolysis of the ester taking place.

In the conversion in vivo of the test substances (see methods) in rats and rabbits, the comparative paper chromatographic studies show that the same metabolites with m-dinitro structure arise (Substance IV/unidentified; Substance V/b-methyl-2-(2-hydroxy-3,5-dinitrophenyl)-propionic acid). The carbonic acid (Substance V) apparently occurs through oxidation of the terminal C of the side chain, while Substance IV probably represents a compound hydroxylated in the side chain. The side chain oxidation suggests the participation of a microsomal oxidase system, which is responsible for the oxidation of aliphatic side chains.

Reduction products (amino compounds) of DNBP or its esters could basically not be demonstrated in the urine of rats but were quite evident in rabbit urine as Substance II (2-sec-butylamino-4,6-nitrophenol). Furthermore, up to 60% of the total excretion in the rabbits was reported as the G-glucuronide (III) of Substance II, but this metabolite was not demonstrated in rat urine. Formation of this product could occur, however, since the glucuronide was found in rat urine when the amino compound (II) was fed to the animals. The authors hypothesized that the first step in the formation of the glucuronide is a reduction of a nitro group, which does not occur in the rats due to inhibition of the nitro group reductase system.

In both species, after a single dose, the quantity of parent compound and both its esters found in the urine within 48 hrs after administration (20') was related to the amount of the substance administered. One exception was the observation of 48% DNBP found in rabbits after a dose of 13.5 mg/kg. Excretion of the parent compounds was greatest during the first 48 hours after administration of DNBP or its esters. However, by the tenth day, around 1% of the amount excreted during the first two days was still occurring.

This report is designated Core Supplementary Data.

005421

STUDY EVALUATION

W. Testan
Secondary Reviewer

| | |
|---|---|
| Study Title: | Disposition of 2-sec-butyl-4,6-dinitrophenol (dinoseb) in pregnant mice |
| Reference: | MRID #39869 |
| Testing Facility: | Department of Pharmacology, Michigan State University, East Lansing, Michigan 48823 |
| Final Report No.: | Food Cosmet. Toxicol. 11: 45-52 |
| Final Report Date: | 1973 |
| Study Authors: | Gibson J.E., Rao K.S. |
| Sponsor: | Dow Chemical Co., NIH Grant ES 00560 |
| Test Material: | Uniformly ring-labelled [$^{14}$C]dinoseb (3.04 mc/m-mole; Mallinkrodt Nuclear Corp., St Louis) mixed with unlabelled dinoseb (Dow Chemical Co., lot # 7200205, 1965) in such a proportion so that in a final concentration, 10 ml/kg gave the appropriate dosage |
| Dose Levels: | 17.7 mg/kg ip or 40 mg/kg orally for colorimetric determination of dinoseb in plasma; 17.7 mg/kg ip and 32 mg/kg per os (each animal receiving approximately 1 uC of radioactivity (25 uC/kg) |
| Species: | Mice, female Swiss-Webster from Spartan Research Animals, Haslett, Michigan |

METHODS:

Paper electrophorograms of blood serum of rats poisoned per os, through skin or by inhalation were examined. The electrophoretic system used differentiates between the presence of albumin-DNPP or albumin-DNBP complexes (colored) and the uncomplexed compounds by their smaller electrophoretic mobility. The metabolites (spots with $R_f$= 0.47-0.51) were detected in the organs of the poisoned animals, mainly in liver and, also, in spleen, kidneys, and blood. The supernatants from these organs and eluates from the chromatograms of metabolites were examined spectrographically (spectrophotometer Unicam SP 500). DNPP and DNBP homogenized with normal rat liver were subjected to incubation in vitro. Further identification of metabolites for possible reduction products were performed. Supernatants of poisoned rat organs and from incubates were subjected to diazotization and coupling with B-naphtol or N-1-naphtylethylenediamine. Brown-red diazo dyes obtained by coupling with B-naphtol were examined spectrophotometrically.

Comments:

This is not a complete study but a letter to the editor. It is obviously not adequate to meet the data requirements necessary for a metabolism study, e.g., lack of detail on the methodologies, lack of individual animal data, lack of test substance characterization, etc.

RESULTS/DISCUSSION/RECOMMENDATIONS:

The absorption spectrum of the supernatants (only liver absorption spectrum shown) from the poisoned rats was compared to the spectrophotometric picture of the supernatants of the incubations from normal rat livers. In both instances the absorption curve in UV proved to be almost identical for DNPP and DNBP in the respective supernatants with a maximum absorption at lambda= approximately 300 mu. Absorption curves for liver supernatants from poisoned rats (per os, skin) and normal livers incubated with DNPP or DNBP revealed a similarity in the spectra obtained with a maximum absorption range of diazocompounds of lambda= 400-460 mu. The authors concluded that the primary amines are the products of the metabolism of DNPP and DNBP and that the enzymatic reduction process of these herbicides mainly occurs in the liver.

This study is classified as Core Supplementary for reasons specified in the methods section.

005121

STUDY EVALUATION

W. Teeters
Secondary Reviewer

| | |
|---|---|
| Study Title: | Metabolites of dinitroisopropylphenol (DNPP) and of dinitrobutylphenol (DNBP) in the rat's organism. A translation of: metabolity dwunitroizopropylofenolu (DNPP) i dwunitrobutylofenolu (DNBP) w organizmie szczurow. |
| Reference: | MRID 60767 |
| Testing Facility: | Department of Toxicological and Forensic Chemistry, Medical Academy, Warsaw |
| Final Report No.: | Acta Poloniae Pharmaceutica XXI(1):222-223 |
| Final Report Date: | 1964 |
| Study Authors: | Herneberg M. |
| Sponsor: | N/A |
| Test Material: | Herbicides DNPP and DNBP |
| Dose Levels: | Unstated |
| Species: | rats |

005421

STUDY EVALUATION

W. Teeters
Secondary Reviewer

| | |
|---|---|
| Study Title: | Effect of food deprivation, phenobarbital, and SKF-525A on teratogenicity induced by 2-sec-butyl-4,6-dinitrophenol (dinoseb) and on disposition of [$^{14}$C]dinoseb in mice* |
| Reference: | N/A |
| Testing Facility: | Department of Pharmacology, Michigan State University, East Lansing, Michigan |
| Final Report No.: | J. Toxicol. and Environ. Health, 1:107-118 |
| Final Report Date: | 1975 |
| Study Authors: | Preache M.M. and Gibson J.E. |
| Sponsor: | N/A |
| Test Material: | Dinoseb obtained from Dow Chemical Co., Midland, Mich., Lot # 7200206, 1966 and [$^{14}$C]dinoseb (uniformly ring labeled, 3.04 mCi/mmol; Mallinckrodt Chemical, St. Louis,Mo) |
| Dose Levels: | 15.8 mg/kg dinoseb and 15.8 mg/kg(25 uCi/kg) [$^{14}$C]dinoseb administered on 10th and 11th day of gestation; [$^{14}$C]dinoseb ( 15.8 mg/kg:25 uCi/kg) administered 1 hr after treatment with 32 mg/kg SKF-525A or after 3 days pretreatment with phenobarbital (50 mg/kg) twice daily; 15.8 mg/kg(25 uCi/kg) after 0, 24 or 48 hr food deprivation or after pretreatment with 40 mg/kg SKF-525A 1 hr before [$^{14}$C] dinoseb or with 50 mg/kg phenobarbital administered twice daily for 3 days |
| Species: | Female mice, Swiss-Webster (Spartan Research Animals, Haslett, Michigan) |

*Teratological considerations are discussed separately.

METHODS:

Three groups of pregnant mice were deprived of food for 0, 24, or 48 hr beginning on the 9th day of gestation. Dinoseb was administered on the 10th and 11th day of gestation(see dose levels, p.1). Three, 6, 12 or 24 hr after [$^{14}$C] dinoseb, blood was obtained by cardiac puncture under ether anesthesia; following centrifugation, 100 ul plasma samples were added to glass scintillation vials. Samples of liver, kidney, lung, placenta, and embryo were collected, minced, added to tared vials, and weighed. Samples were solubilized in 1.0 ml Soluene, 15 ml toluene counting solution (5 gm PPO/diphenyloxazole, plus 200 mg dimethyl POPOP/1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene per liter toluene) added and radioactivity levels measured with a liquid scintillation counter and expressed as dpm/ul plasma or mg tissue.

In a second experiment, two groups of nonpregnant mice were treated with [$^{14}$C]dinoseb (see p.1) after 1 hr pretreatment with SKF-525A or after 3 days pretreatment with phenobarbital twice daily. A third group was not pretreated. Samples of blood, liver, kidney, and lung were obtained 3, 6, 12, or 24 hr after the radiolabeled dinoseb; plasma was separated by centrifugation, and radioactivity determined in plasma and tissue by the method described above.

Five groups of nonpregnant mice were treated with [$^{14}$C]dinoseb (see p.1) after 0, 24, or 48 hr food deprivation or after pretreatment with SKF-525A 1 hr before [$^{14}$C]dinoseb or with phenobarbital twice daily for 3 days. Blood and liver samples were collected 3 or 24 hr later, plasma was separated, and aliquots prepared to determine total radioactivity as described above. Dinoseb metabolites were separated from parent compound by methyl ethyl ketone extraction and radioactivity in the extract determined as described in Gibson and Rao (Food Cosmet. Toxicol. 11:45-52, 1973). The method was shown by thin-layer chromatography to extract only unmetabolized dinoseb with a recovery of 99% in plasma.

For determination of % body water after food deprivation, nonpregnant mice were killed by cervical dislocation after 0, 24 or 48 hr food deprivation. Body weight was determined after removal of the skin and tail; the body was homogenized in an equivalent weight of distilled water. Three aliquots of the homogenate from each mouse were weighed and dried to a constant weight. Data were expressed as the mean per cent body water for the three aliquots.

Comparison of the rate of disappearance of [$^{14}$C]dinoseb were made by a t test for the difference between slopes (Goldstein, 1964). All other data were studied by analysis of variance using between-group comparisons by the least significant difference test (Steel and Torrie, 1960).

Comment:

This study, obtained from the open literature, was not designed to meet the standards for a metabolism test to be submitted for EPA's regulatory requirements but contributes to our scientific understanding of dinoseb's metabolism.

RESULTS:

The authors reported the disappearance of labeled dinoseb from maternal plasma and tissues (liver, kidney, embryo, placenta, lung) to be first order kinetics. Food deprivation for 24 hr prior to administration of the label significantly reduced the rates of radioactivity elimination from the maternal plasma and lung, while food deprivation for 48 hr increased the rate of label disappearance from liver. The embryo had lower levels of [$^{14}$C]dinoseb as compared with those in the maternal plasma or tissues, regardless of the deprivation conditions. The rate of disappearance of radiolabel was reported as not significantly altered by food deprivation of the mother.

In nonpregnant mice, phenobarbital pretreatment increased the rate of radioactivity elimination from plasma and tissues, e.g., no pretreatment: $t_{1/2}$=10.75 hr vs. phenobarbital pretreatment: $t_{1/2}$=4.00 hr. SKF-525A pretreatment significantly decreased the rate of disappearance of dinoseb radioactivity from the liver ($t_{1/2}$= 12.00 hr vs. 10.00 hr in no pretreatment group) but not from plasma or other tissues.

Radioactivity distribution is given below in a table. In a previous study (Gibson and Rao, Food Cosmet. Toxicol. 11:45-52, 1973), it was reported that 100% of the radioactivity in plasma of mice treated with [$^{14}$C]dinoseb was in the form of parent compound. There were no statistically significant alterations in the percentage of unmetabolized parent compound in the plasma with any pretreatment. Forty-eight hour food deprivation resulted in a significant decrease in the levels of radiolabel in the 24 hr sample (post-administration) of liver but 24 hr deprivation had no effect on any parameter measured. SKF-525A significantly increased the total radioactivity found in the liver at 24 hours after compound administration whereas phenobarbital significantly decreased the total radiolabel at both 3 and 24 hours postadministration (plasma and liver) and significantly increased the conversion of the parent compound to metabolites in the 24 hour liver sample ($p<0.05$).

The authors reported that food deprivation had no effect on percent body water. The mean percentage body water for groups deprived for 0, 24 and 48 hr was 66, 67 and 61%.

DISCUSSION/RECOMMENDATIONS:

The biodisposition study was performed concomitant with a teratology study in which teratogenic/fetotoxic effects of dinoseb administered i.p. appeared to be modified (enhanced, no effect) by food deprivation for 24 hours but not for 48 hours, respectively. The mechanism of this effect may relate to altered metabolism.

The enhanced teratogenic/fetotoxic effect of dinoseb i.p. after 24 hrs of food deprivation appeared related to a decrease in the compounds' metabolism as evidenced by a decrease in the rate of elimination of radiolabel from the plasma of the dams whereas the rate of disappearance at 48 hr of food deprivation increased in the liver suggesting an increased metabolic activity of the liver at that time period.

The toxicity of dinoseb (teratogenicity/fetotoxicity) could also be alleviated by stimulation of liver microsomal metabolism through pretreatment with phenobarbital, or enhanced by the administration of SKF-525A through inhibition of liver microsomal metabolism.

(table taken from table 4, page 116 of study)

[Nonpregnant]

| time after [$^{14}$] dinosb. | total radioactivity[a] plasma 3 | plasma 24 | liver 3 | liver 24 | % radioactivity in parent compound form[b] plasma 3 | plasma 24 | liver 3 | liver 24 |
|---|---|---|---|---|---|---|---|---|
| no pretrmt. | 267(14) | 51(8) | 92(8) | 18(1) | 104(4) | 101(2) | 60(6) | 54(4) |
| 24-hr depriv. | 244(4) | 65(9) | 102(6) | 17(0) | 98(4) | 104(3) | 65(2) | 64(8) |
| 48-hr depriv. | 303(12) | 58(10) | 87(1) | 12(1)* | 106(4) | 62(3) | 61(3) | 61(4) |
| SKF-525A | 288(8) | 67(8) | 84(2) | 27(1)* | 98(6) | 92(2) | 70(3) | 57(3) |
| phenobarb. | 146(15)* | 5(1)* | 82(3) | 7(2)* | 103(1) | 78(6) | 52(11) | 12(6)* |

[a] dpm/mg/tissue or ul plasma (SE); [b] MEK extractable radioactivity/total radioactivity (SE); significantly different from no pretreatment group ($p<0.05$)

In nonpregnant mice, phenobarbital pretreatment increased the rate of radioactivity elimination from plasma and tissues, e.g., no pretreatment: $t_{1/2}$=10.75 hr vs. phenobarbital pretreatment: $t_{1/2}$ =4.00 hr. SKF-525A pretreatment significantly decreased the rate of disappearance of dinoseb radioactivity from the liver ($t_{1/2}$= 12.00 hr vs. 10.00 hr in no pretreatment group) but not from plasma or other tissues. Forty-eight hour deprivation resulted in a significant decrease in the levels of radiolabel in the 24 hr sample(post-administration) of liver but 24 hr deprivation had no effect on any parameter measured. None of the pretreatments altered the percentage of unmetabolized parent compound in the plasma. Although metabolic activity may not be identical in the pregnant and nonpregnant state in mice, Gibson and Rao (Fd. Cosmet. Tox. 11:45-52, 1973) had previously shown that the disappearance of dinoseb from the plasma of pregnant and nonpregnant female mice was not different.

Food deprivation did not alter the percent body water, which could be hypothesized to affect dinoseb's embryotoxicity through altering the volume of distribution and thus the concentration of dinoseb in the embryo.

It is concluded that alteration of the environmental conditions (food deprivation for 24 or 48 hrs) either enhances or has no effect on the teratogenic or fetotoxic effects of dinoseb administered ip to Swiss-Webster mice during days 10-12 of gestation. This appears to occur through a decrease in the metabolic inactivation of dinoseb with 24 hrs of food deprivation and an increase in metabolism after 48 hr food deprivation as evidenced by a reduction in the rate of disappearance of plasma radioactivity or an increased rate of disappearance in the liver, respectively. Administration of SKF-525A also enhances the toxicity of dinoseb while phenobarbital decreased it. These effects are mediated through either an inhibition of metabolism of the dinoseb or an enhanced metabolic effect in the liver. Evidence in the nonpregnant mice supports this finding. The altered embryotoxicity from food deprivation is not apparently due to an altered volume of distribution since food deprivation had no effect on the per cent body water.

This study is designated as Core Supplementary.

APPENDIX VI: FINDINGS OF THE TOXICOLOGY BRANCH PEER REVIEW COMMITTEE
ABBREVIATED REVIEW

The Toxicology Peer Review Committee met for an abreviated peer review of the oncogenic potential of Dinoseb on May 28, 1986. After a review of the data base, it was tentatively concluded that Dinoseb is a Class C oncogen based on the positive liver tumor livers in female mice. Primary data required to further define the oncogenic potential of Dinoseb are a 2-year rat oncogenicity study, mutagenicity studies which include gene mutation tests in mammalian cells and a chromosomal aberration test, as well as a general metabolism study. Secondary data requirements are a 1-year dog study, a 2-generation reproduction study and tests in two species for teratology.

METHODS:

Mice were housed in groups of ten in stainless-steel cages and allowed food and water ad lib. A 12-hr dark/light cycle was maintained (lights on at 8 a.m.). Pregnant mice were obtained by daily pen breeding from 8 to 9 a.m. Copulation was confirmed by the presence of the vaginal plug at examination. This time was called day 1 of gestation.

Groups of five adult female mice were given dinoseb ip or orally. Fresh aqueous solutions of dinoseb (dissolved in 1 N NaOH and titrated to neutral pH with 1 N-HCl) were prepared so that 10 ml/kg gave the desired dosage. At various times (0.03-24hr) after administration, the mice were lightly anesthetized with ether and blood was collected by cardiac puncture into heparinized syringes. After centrifugation of the blood, plasma samples (0.4-0.5 ml) were diluted with distilled water to 5 ml and mixed. After addition of 5 ml methyl ethyl ketone (MEK) and 1 g NaCl-$Na_2CO_3$ (9:1, w/w), the tubes were shaken. The MEK phase was withdrawn and the MEK extraction was repeated. The combined MEK extracts were then read at 430 nm against dinoseb standards (0-10 ug/ml).

Radioactive dinoseb(see pg. 1 for details) was mixed with cold dinoseb proportionately so that, in the final concentration, 10 ml/kg gave the correct dosage. At various times (1 min-48 hr) after administration of [$^{14}$C]dinoseb, samples of maternal blood, liver, kidney, brain, muscle and adipose tissue were collected. In addition, samples of embryo, placenta, and uterus were taken. Embryos and placentae from each animal were pooled. Samples were minced with scissors, transfered to tared glass liquid-scintillation vials and weighed. Plasma samples (100 ul) were placed in separate liquid scintillation vials. All samples were solubilized in 1.0 ml Soluene 100® and 15 ml toluene counting solution (5 g PPO plus 200 mg dimethyl POPOP/liter toluene) was added. Radioactivity levels were measured with a liquid scintillation counter. Quench corrections were made using [$^{14}$C]toluene internal standardization. Disintegrations/min/g tissue were converted to ug dinoseb/g tissue to eliminate the effect of differences in the specific activities of injected dinoseb.

The kinetic model of Wiegand and Sanders (1964) was used to determine the rate constants for dinoseb absorption ($k_a$) and elimination ($k_e$) from the plasma levels of the compound. Also, using the kinetic model, the volume of distribution was estimated.

The radioactivity of urine and feces collected for periods of 1-64 hr after oral or ip administration of [$^{14}$C]dinoseb was determined in groups of non-pregnant mice treated as described above. Three groups of 3 mice were used for each sampling time. Weighed fecal samples were homogenized in 2 volumes of 1 N HCl and aliquots of the fecal homogenate were transferred to liquid scintillation vials. Feces and urine samples were solubilized and counted as described above. Excretion was expressed as a cumulative percentage of the administered dose for each time period. In other experiments the common bile duct of female mice, anesthetized with 60 mg pentobarbital/kg, was cannulated using polyethylene tubing. The abdominal incision was closed and dinoseb was administered ip or by stomach injection and bile was collected into tared scintillation vials. The bile was weighed, solubilized and counted for radioactivity as described.

[$^{14}$C]Dinoseb was separated from its metabolites in blood, liver, kidney and

embryo tissues. Tissues were collected 3 hr after ip or oral administration of the compound to pregnant mice on day 11 of gestation. The MEK extraction procedure already described was used, with the following modifications. Tissues were homogenized in distilled water (1 g in 4 ml) and one 10 ml MEK extraction was used. An aliquot of the MEK extract was counted for radioactivity. The remaining MEK was evaporated to 0.1 ml under nitrogen and spotted on cellulose thin-layer chromatography (TLC) plates. MEK extracts were chromatographed against standard [$^{14}$C]dinoseb using two solvent systems; n-butanol-ethanol-benzene-$NH_4OH$ (2:4:2:2, by vol.) and n-butanol-acetic acid-$H_2O$ (6:2:2, by vol.). Radioactivity on the TLC plates was located with a radiochromatogram scanner. Only dinoseb was extracted in MEK (recovery>99%).

Statistical analyses of data were made by the grouped t test (Steel and Torrie, 1960). The level of significance was $p<0.05$.

Comments:

This study was obtained from the open literature and was not designed to meet the regulatory requirements for a metabolism study.

RESULTS:

The authors reported that the administration of dinoseb ip in a dose of 17.7 mg/kg to female mice produced a colorimetric peak in the blood comparable with an oral dose of 40 mg/kg. For subsequent studies the authors chose an ip dose of 17.7 mg/kg and an oral dose of 32 mg/kg, since these dose levels produced, respectively, teratogenic and essentially non-teratogenic effects when given repeatedly during gestation.

Oral or ip administration of [$^{14}$C]dinoseb resulted in placental transfer of the material to the embryo but the levels never exceeded 2.5% indicating a placental barrier. Both routes of administration gave similar embryonic levels but the peak levels were reached much more rapidly from the ip route (within 8 minutes) compared to the oral route (12 hr). The radioactivity reached all tissue although there was evidence of a blood brain barrier (brain radiolabel was of the same magnitude as the embryo). Radioactivity was reported in all tissues to be at levels between those in the plasma and embryo, except in the case of the liver, which had initially higher levels of radioactivity than the plasma.

Table 1: Pharmacokinetics of [$^{14}$C]dinoseb (taken from Table 1, p.48 of report)

| route of admin. | dose(mg/kg) | $k_a(hr^{-1})$ | $k_e(hr^{-1})$ | volume of distribution (liters/kg) |
|---|---|---|---|---|
| oral intubation | 32 | 7±4 | 0.02±.01 | 0.66±.13* |
| ip injection | 17.7 | 299±205 | 0.09±.02 | 0.18±.18† |

*volume of distribution indicates distribution to total body water; † volume of distribution indicates distribution to extracellular water; values are means for groups of five mice ± SEM; $k_a$= absorption constant, $k_e$= elimination constant

In Table 1 (above) the rates of absorption and excretion are given. Ip injection resulted in a nearly 43-fold faster absorption of the [$^{14}$C]dinoseb than did oral administration. The rate of elimination was roughly 4-fold greater for radiolabel administered ip than orally, while the volume of distribution was different—the oral route resulted in a distribution to total body water as opposed to distribution only to the extracellular water with the ip route.

Table 2: Excretion of [$^{14}$C]dinoseb (taken from Table 2, p. 49 of report)

| time after administration (hr) | mean cumulative excretion (% administered radioactivity) | | | | | |
|---|---|---|---|---|---|---|
| | bile | | urine | | feces | |
| treatmt: mg/kg : | oral 32 | ip 17.7 | oral 32 | ip 17.7 | oral 32 | ip 17.7 |
| 0.5 | 0.1±0 | 0.2±0.1 | --- | --- | --- | --- |
| 1 | 0.4±0.1 | 0.6±0.1 | 0.8±0.2 | 1.4±0.2 | --- | --- |
| 2 | 0.6±0.3 | 1.4±0.4 | 1.9±0.4 | 3.9±0.1 | --- | --- |
| 4 | 0.9±0.4 | 3.9±0.6 | 3.2±0.4 | 7.0±0.1 | --- | --- |
| 8 | 1.4±0.6 | 9.6±1.4* | 6.8±1.4 | 13.4±1.3 | 0.5±0 | 3.3±0.9 |
| 16 | --- | --- | 14.4±2.0 | 22.1±2.1 | 4.3±1.1 | 11.1±1.1* |
| 32 | --- | --- | 2[illegible].2±3.5 | 26.3±1.9 | 9.7±3.7 | 28.7±4.8* |
| 64 | --- | --- | 26.3±3.3 | 28.2±2.5 | 30.4±7.5 | 40.8±6.5 |

values are means for groups of three mice ± SEM; * significantly different from corresponding value for orally treated animals ($p<0.05$)

Different $k_e$ for the two routes of administration were due to the mode of excretion (see Table 2 above), which was significantly different for the two routes with the radiolabel from the ip route being more rapidly excreted in the bile and subsequently in the feces as compared to the orally administered material. Cumulative mean urinary excretion was similar for the two routes.

Table 3: Separation of dinoseb from its metabolites 3 hr after oral or ip administration to pregnant mice on day 11 of gestation(taken from Table 3, p. 50 of report)

| tissue | treatment: dose(mg/kg): | mean tissue levels(ug/g)† of dinoseb and metabolites | | | |
|---|---|---|---|---|---|
| | | total¶ | | dinoseb alone§ | |
| | | oral 32 | ip 17.7 | oral 32 | ip 17.7 |
| blood | | 31.3±5.4 | 45.0±1.4* | 29.7±4.6 | 46.6±3.9 |
| liver | | 26.5±4.1 | 32.9±1.3 | 14.2±0.6(54%) | 15.6±1.8(47%)[a] |
| kidney | | 21.8±3.9 | 28.6±4.4 | 13.8±1.4(63%) | 15.4±1.4(48%) |
| embryo | | 2.1±0.2 | 5.1±0.4* | 1.8±0.1( 9%) | 2.9±0* (57%) |

values are means for groups of at least three animals±SEM; † based on the molecular weight of dinoseb; ¶ measured by total radioactivity in samples; § measured as MEK-extractable material; * significantly different from the corresponding value for orally treated animals($p<0.05$); [a] % total radioactivity

Parent compound and metabolite profiles (see Table 3 above) indicate that dinoseb (3 hr after administration) in pregnant mice (via either route) is basically unmetabolized parent compound in the blood but not in the liver, kidney, and embryo (ip injection only) where the ip route indicates a somewhat greater metabolic conversion (47, 48 and 57%, resp.) of the parent material than does the oral route (54, 63, and 9%, resp.). The identity of the metabolites was not determined.

DISCUSSION/RECOMMENDATIONS:

The teratogenic and embryotoxic properties of dinoseb were reported by the authors to be dependent on the route of administration— ip injection producing toxicity, oral intubation producing no effects (Gibson, Toxicol. 11:31, 1973). This difference may relate to the much more rapid absorption and distribution of dinoseb when it is administered via the ip route--peak levels were observed at 8 minutes after ip injection as opposed to peak levels at 12 hr after the oral intubation. It should be noted that peak levels were essentially the same via both routes. This would allow for the embryo to be exposed more quickly and for a more prolonged time by ip than via the oral route. There was no difference noted in the total uptake of radioactivity in the embryo after 12 hrs or so (2.5%) via either route but dinoseb was more quickly eliminated (4-fold faster) thru an increase in biliary excretion (reported as significantly different at 8 hours after administration) and loss through the feces following the ip than the oral route.

The differential toxicity may relate to intestinal microbial metabolism of the orally administered material as opposed to no microbial metabolism via the ip route. Metabolism has been reported in the rabbit, rat and various ruminants. Rabbits, but not rats, reduced one nitro group of dinoseb with the formation of the 6-amino derivative conjugated as the O-glucoside. A carboxylic acid was also formed by oxidation of the terminal carbon of the secondary side chain in both rats and rabbits. In rumen fluid, dinoseb (under anerobic conditions) was converted to the 6-amino derivative, with successive reduction to the diamino compound. This reduction did not take place in the heat sterilized rumen. In vivo, the diamino compounds were not demonstrated in blood plasma in the cow. Metabolic conversion of parent compound in the pregnant mice appeared to be roughly equal via both routes (50%) except for the embryo where the radioactivity of the metabolite was higher in the ip injected animals ( 57%) as opposed to the orally-treated mice (9%). This would suggest that the unknown metabolites in the embryo might account, in part, for the differential effects seen.

This study is designated as Core Supplementary data.

# APPENDIX V: PUBLISHED TOLERANCES

| Commodity | Parts per million |
|---|---|
| Barley, meal | 0.1 (N) |
| Sheep, fat | 0.1 (N) |
| Sheep, mbyp | 0.1 (N) |
| Sheep, meat | 0.2 (N) |
| Soybeans | 0.2 (N) |
| Soybeans, forage | 0.2 (N) |
| Wheat, grain | 0.2 (N) |
| Wheat, straw | 0.2 (N) |

[43 FR 29121, July 6, 1978]

§ 180.280 Dimethyl phosphate of α-methylbenzyl 3-hydroxy-*cis*-crotonate; tolerances for residues.

Tolerances are established for negligible residues of the insecticide dimethyl phosphate of α-methylbenzyl 3-hydroxy-*cis*-crotonate in meat, fat, and meat byproducts of cattle, goats, hogs, and sheep and in milk at 0.02 part per million.

§ 180.281 Dinoseb; tolerances for residues.

(a) Tolerances are established for residues of the herbicide, insecticide, and fungicide dinoseb (2-*sec*-butyl-4,6-dinitrophenol) from application of its phenol or its readily hydrolyzable salts (alkanolamine salts, ammonium salt, or sodium salt) in or on the following raw agricultural commodities:

| Commodities | Parts per million |
|---|---|
| Alfalfa | 0.1(N) |
| Alfalfa, hay | 0.1(N) |
| Almonds | 0.1(N) |
| Almonds, hulls | 0.1(N) |
| Apples | 0.1(N) |
| Apricots | 0.1(N) |
| Barley, forage | 0.1(N) |
| Barley, grain | 0.1(N) |
| Barley, straw | 0.1(N) |
| Beans | 0.1(N) |
| Beans, forage | 0.1(N) |
| Beans, hay | 0.1(N) |
| Birdsfoot, trefoil | 0.1(N) |
| Birdsfoot, trefoil, hay | 0.1(N) |
| Blackberries | 0.1(N) |
| Blueberries | 0.1(N) |
| Boysenberries | 0.1(N) |
| Cherries | 0.1(N) |
| Citrus | 0.1(N) |
| Clover | 0.1(N) |
| Clover, hay | 0.1(N) |
| Corn, fodder | 0.1(N) |
| Corn, forage | 0.1(N) |
| Corn, fresh (inc. sweet K + CWHR) | 0.1(N) |
| Corn, grain (inc. popcorn) | 0.1(N) |
| Cotton, forage | 0.1(N) |
| Cottonseed | 0.1(N) |
| Cottonseed, hulls | 0.1(N) |
| Cucurbits | 0.1(N) |
| Currants | 0.1(N) |
| Dates | 0.1(N) |
| Figs | 0.1(N) |
| Filberts | 0.1(N) |
| Garlic | 0.1(N) |
| Gooseberries | 0.1(N) |
| Grapes | 0.1(N) |
| Hops | 0.1 |
| Lentils | 0.1(N) |
| Loganberries | 0.1(N) |
| Nectarines | 0.1(N) |
| Oats, forage | 0.1(N) |
| Oats, grain | 0.1(N) |
| Oats, straw | 0.1(N) |
| Olives | 0.1(N) |
| Onions | 0.1(N) |
| Peaches | 0.1(N) |
| Peanuts | 0.1(N) |
| Peanuts, forage | 0.1(N) |
| Peanuts, hay | 0.1(N) |
| Peanuts, hulls | 0.1(N) |
| Pears | 0.1(N) |
| Peas | 0.1(N) |
| Peas, forage | 0.1(N) |
| Peas, hay | 0.1(N) |
| Pecans | 0.1(N) |
| Plums (prunes) | 0.1(N) |
| Potatoes | 0.1(N) |
| Raspberries | 0.1(N) |
| Rye, forage | 0.1(N) |
| Rye, grain | 0.1(N) |
| Rye, straw | 0.1(N) |
| Soybeans | 1.0 |
| Soybeans, forage | 1.0 |
| Soybeans, hay | 1.0 |
| Strawberries | 0.1(N) |
| Vetch | 0.1(N) |
| Vetch, hay | 0.1(N) |
| Walnuts | 0.1(N) |
| Wheat, forage | 0.1(N) |
| Wheat, grain | 0.1(N) |
| Wheat, straw | 0.1(N) |

(b) Tolerances are established for residues of the herbicide, insecticide, and fungicide dinoseb (2-*sec*-butyl-4,6-dinitrophenol) from application of its phenol or its readily hydrolyzable salts (ammonium salt or sodium salt) in or on the following raw agricultural commodities:

| Commodities | Parts per million |
|---|---|
| Grasses, pasture | 0.1 |
| Grasses, pasture, hay | 0.1 |

(Sec. 408(e), 68 Stat. 514 (21 U.S.C. 346(a)(e)))

[48 FR 9004, Mar. 3, 1983, as amended at 49 FR 21711, May 23, 1984]

cf 180.251 [illegible] 7/88

005421

File last updated 10/11/..

[illegible] Grant Information

| Chronic (LOEL) mg/kg/day | Acute ppm | U.F. | mg/kg/day | ug/kg(50kg) |
|---|---|---|---|---|
| 1.000 | 20.00 | 3000 | 0.003 | 0.2 |

NOEL change not recorded ssr

Published Tolerances

| Crop | Tolerance ppm | Food Factor | mg/kg(1.5kg) |
|---|---|---|---|
| Almonds( 1) | 0.100 | 0.03 | 0.[illegible] |
| Apples( 2) | 0.10 | 0.33 | 0.[illegible] |
| Apricots( 3) | 0.10 | 0.1 | 0.[illegible] |
| Barley( 4) | 0.10 | 0.03 | 0.[illegible] |
| Beans( 5) | 0.20 | 0.07 | 0.[illegible] |
| Blackberries( 16) | 0.10 | 0.03 | 0.[illegible] |
| Blueberries( 14) | 0.10 | 0.03 | 0.[illegible] |
| Boysenberries( 17) | 0.10 | 0.03 | 0.[illegible] |
| Cherries( 20) | 0.10 | 0.1 | 0.[illegible] |
| Citrus fruits( 33) | 0.20 | 0.41 | 0.[illegible] |
| Corn,grain( 34) | 0.10 | 1.0 | 0.[illegible] |
| Corn,pop( 35) | 0.10 | 0.3 | 0.[illegible] |
| Corn,sweet( 36) | 0.10 | 1.43 | 0.[illegible] |
| Cottonseed (oil)( 42) | 0.10 | 0.15 | 0.[illegible] |
| Dewberries( 45) | 0.20 | [illegible] | 0.[illegible] |
| Currants( 49) | 0.10 | 0.03 | 0.[illegible] |
| Grapes( 51) | 0.10 | 0.3 | 0.[illegible] |
| Figs( 57) | 0.10 | 0.03 | 0.[illegible] |
| Filberts( 58) | 0.10 | 0.03 | 0.[illegible] |
| Garlic( 61) | 0.10 | 0.03 | 0.[illegible] |
| Gooseberries( 63) | 0.10 | 0.03 | 0.[illegible] |
| Hops,dried cones( 66) | 0.10 | 0.03 | 0.[illegible] |
| Okra( 75) | 0.10 | 0.03 | 0.[illegible] |
| Raspberries( 93) | 0.10 | 0.03 | 0.[illegible] |
| Rhubarb(102) | 0.10 | 0.03 | 0.[illegible] |
| Olives(104) | 0.10 | 0.03 | 0.[illegible] |
| Onions(105) | 0.10 | 0.03 | 0.[illegible] |
| Peaches(116) | 0.10 | 0.03 | 0.[illegible] |
| Peanuts(117) | 0.10 | 0.03 | 0.[illegible] |
| Pears(119) | 0.10 | 0.03 | 0.[illegible] |
| Peppers(120) | 0.1 | 0.03 | 0.[illegible] |
| Plums (prunes)(126) | 0.10 | 0.03 | 0.[illegible] |
| Prunes(127) | 0.10 | 0.03 | 0.[illegible] |
| Rye(130) | 0.10 | 0.03 | 0.[illegible] |
| Safflower (oil)(134) | 0.10 | 0.03 | 0.[illegible] |
| Strawberries(152) | 0.10 | 0.03 | 0.[illegible] |
| Walnuts(157) | 0.10 | 0.03 | 0.[illegible] |
| Wheat(159) | 0.10 | 0.03 | 0.[illegible] |
| Youngberries(163) | 0.10 | 0.03 | 0.[illegible] |
| Zucchini( 167) | 0.10 | 0.03 | 0.[illegible] |